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Investigating factors affecting the future survival of Exmoor ponies: implications for ecological restoration and management of the breeding population

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Exmoor pony grazing on Scoraig. Photo: Erik Kusch

This thesis is submitted in fulfilment of the requirements for the degree of Doctor of Philosophy.

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Abstract

The current global climate and biodiversity crises are interlinked. Top-down trophic rewilding, using large herbivores, may make an important contribution to the restoration efforts needed to mitigate the global biodiversity crisis and increase ecosystems capacity to mitigate climate change. Exmoor ponies, a rare breed, are widely used in this role, as ecological engineers, on conservation grazing sites and in rewilding projects.

The overall purpose of this study was to investigate factors affecting the future survival of the Exmoor pony population, including the implications of their role in ecological restoration and rewilding. Since most Exmoor ponies are bred in free-living herds, it is expected that the continual exposure to environmental pressures increases selection of natural survival fitness traits. The specific aims of the study were to: 1) investigate the effects of grazing manipulations, using Exmoor ponies, on biodiversity and flowering plant abundance; 2) assess the impact of parasite load and seasonality on body condition in a herd of free-living Exmoor ponies; and 3) combine pedigree and molecular data to inform conservation breeding management of the Exmoor pony population.

In ecological restoration projects, herbivores are used to manipulate biodiversity, sometimes with the aim of increasing or decreasing the abundance of particular species. There is considerable anecdotal evidence of the positive impact of Exmoors grazing from established projects, but quantification of the results of their impact, and of their impact in comparison to other species of large herbivores would improve information for conservation managers. To investigate the impact of different grazing treatments the study described in Chapter 2, I used exclosures, enclosures, unmanipulated grasslands, and areas grazed exclusively for >30 years by Exmoor ponies or commercial livestock, to test whether the effects of different grazing treatments impacted overall vegetative biodiversity and, specifically, flowering plant abundance. The long-term grazed plots were used as an indicator of the stabilised effects of grazing by different large herbivore species. I found that Exmoor grazing positively impacted biodiversity and increased the abundance of flowering plants in comparison to the other treatments, including grazing by commercial cattle and sheep. A dynamic Bayesian model suggested the changes resulting from Exmoor grazing would be persistent. These results support anecdotal evidence from conservation grazing projects about the positive impact of Exmoors' grazing and that they can be used as eco-engineers to improve overall biodiversity and flowering plant abundance.

Animals on restoration or rewilding sites rarely receive routine anthelmintic treatment or supplementary feeding in winter, so need to be resistant to the challenges of parasite loads, and of seasonal variation in vegetation quality and abundance, combined with the increased energy demands of winter weather. Seasonal variation in weather and changes in the availability of food resources, and gastro-intestinal parasite load can negatively impact the body condition of free-living horses. In Chapter 3 I assessed whether this low intervention management associated with restoration or rewilding projects could have impacts on body condition which could compromise the welfare of Exmoors in rewilding. Body condition scoring and faecal egg counts were performed in spring and autumn to investigate the impact of seasonality and parasite load on the body condition of free- living Exmoor ponies, not receiving supplementary feeding, or treated with anthelmintics. While winter resource limitation negatively correlated with body condition scores, individuals consistently gained condition in summer. Whilst parasite load was negatively correlated with body condition, parasite loads were generally lower than reported in other studies of free-living horses. Parasite load capacity is shown to be moderately heritable in Sable Island feral horses. If further investigation verified the comparatively lower parasite loads in Exmoors, this trait could be positively selected.

Exmoors are known to have suffered a genetic bottleneck of <50 individuals in the 1940s. Genetic bottlenecks are likely to be followed by increased inbreeding and genetic load. As most Exmoors are bred in free-living herds, pedigree verification by observation is not always reliable. Accurate identification of post-bottleneck founder lines could facilitate targeted breeding to prevent further loss and improve even representation of founders across the population. In Chapter 4 I combined Stud Book records with mtDNA and whole genome sequencing (WGS) to verify matrilines, estimate inbreeding based on runs of homozygosity (ROH) and investigate deleterious allele frequencies in relation to inbreeding. Using DNA extracted from hair samples from animals selected to represent a broad cross-section of the UK founder bloodlines, we aimed to get a representative view of the extant population. mtDNA haplotype networks revealed good overall congruence with recorded matrilines. Stud Book records revealed that representation of the founder lines within the population was uneven. Investigation of ROHs showed Exmoors to have varying, but mostly not significantly different historic and current levels of inbreeding in comparison with 23 other breeds. The proportion of loss of function to synonymous derived alleles declined significantly with increasing 10Mb ROH values, possibly indicating purging of deleterious alleles. These results improve stud book record accuracy, and understanding of inbreeding and relatedness and can therefore inform targeted conservation breeding to safeguard the future population.

Exmoor ponies need a role to ensure their future survival and linking that role to the preservation of their habitat will increase their conservation value and therefore population viability. The future of the Exmoor pony population is likely to be linked with their role as ecological engineers. This role may be crucial to their survival in a world threatened by the crises of biodiversity loss and climate change, as their value in contributing to biodiversity gains and to climate change mitigation is increasingly understood and appreciated. It is likely that a large proportion of the Exmoor population will continue to be born into free-living herds, where they will continue to be subject to natural, environmental selection pressures, increasing the likelihood that they will retain the survival and ecological interaction traits needed for them to thrive when rewilded. Ongoing monitoring of the founder representation, inbreeding and deleterious alleles could inform breeding so that a healthy, viable population can be maintained.

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Feith an Fheoir. Photo: Debbie Davy

Author's declaration

I declare that the work recorded in this thesis is entirely my own, except where otherwise stated, and that it has not been submitted as part of a degree elsewhere. Much of this thesis has been produced in collaboration with others and their contributions are listed below.

Chapter 1: Appendix 1: Table A1, prepared by Sue Baker. Detailed comments on drafts were provided by Barbara Mable (BM) and Jason Matthiopoulos (JM).

Chapter 2: The Principal Component Analysis was created by Aud Helen Halbritter. BM created other RMarkdown script. The Bayesian predictive model was created by JM. Detailed comments on drafts were provided by BM and JM.

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Chapter 5: Detailed comments on drafts were provided by BM and JM.

Chapter 1: Introduction

1.1 The climate and biodiversity crises

The appearance of humans coincided with the time of the greatest biodiversity on Earth and the increasing dominance of the human population has corresponded to the reduction of this biodiversity to its lowest level since the Mesozoic era 65 million years ago, an extreme decline in biodiversity (Wilson 1989). Complex ecosystems evolve over millions of years, but since the rapid spread of humans, super-predators, across the globe many megafauna species either became extinct or underwent drastic reductions in their populations (Malhi et al. 2016). Species extinction rates are such that the Earth may be in the 6th Mass Extinction period in its 4.5-billion-year history (Ceballos et al. 2015). Although, particularly in the North American context, climate change has been considered a contributory factor to these late-Pleistocene extinctions, recent investigation of both ancient climate dynamics and human expansion indicates that this decline is likely due to the global expansion of the human population rather than just climate dynamics, with the timing of the spread of humans to different continents coinciding with mass extinctions irrespective of the prevailing regional climate (Bergman et al. 2023; Malhi et al. 2016). Genomic studies of 139 extant megafauna species indicate population declines in 91% of all species throughout the Quaternary period, the greatest impact of this global decline being observed in megafauna populations (Bergman et al. 2023). The ‘keystone herbivore’ hypothesis, extrapolated from observation of the grazing habits of extant African megaherbivores, proposed that their influence created spatially diverse vegetation patterns favouring the abundance of smaller herbivores dependent upon the diversity of the habitat for nutrition and shelter, and thus proposed that the elimination of megaherbivore influence was the major factor leading to habitat changes at the end of the Pleistocene period (Owen-Smith 1987). Of the 74 surviving species of megaherbivores (≥100 kg body mass), 59% are currently threatened with extinction (Svenning 2020). The loss of these megafauna corresponds to reductions in functional diversity: complex megafauna populations promote landscape-scale biodiversity, by generating environmental heterogeneity such as varied vegetation structure, created by a mixture of grazers and browsers, and by their role in the dispersal of propagules such as seeds (Svenning 2020). The loss of the ecological services provided by megaherbivores may result in cascading effects on other species (including large carnivores, scavengers, and meso-herbivores), and can thus result in enormous ecological consequences (Ripple et al. 2015). This historic loss of the species that drove the creation of large-scale biodiverse communities continues into the current Anthropocene Era (Svenning 2020).

That we are currently facing a global decline in biodiversity crisis is now widely recognised (Isbell et al. 2023). The ongoing drivers of this crisis are: habitat loss, as human-induced changes in land use reduce or fragment habitats; pollution damaging habitat quality; overexploitation of natural resources beyond their regenerative capacity; competition from invasive species; and climate change, which can affect species range and viability (Isbell et al. 2023). These may act synergistically and the actual rate of biodiversity loss and the threats to ecosystems may not be accurately quantified (Isbell et al. 2023). The UN Decade of Ecological Restoration, 2021-2030, and the 30 x 30 initiative, part of the Kunming-Montreal Global Biodiversity Framework (GBF), was adopted at the Conference of the Parties (COP15) meeting, under the auspices of the United Nations Framework Convention on Climate Change (UNFCCC), which aims to protect at least 30% of land, rivers, lakes, wetlands and sea by 2030, all aim to protect and restore ecosystems and thus also address the biodiversity losses by protecting habitats. The Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES) recognises the inter-relationship between biodiversity, ecosystem services and climate change and seeks to advise on policies that address these problems (Díaz et al. 2015).

1.2 Shifting baselines and reference ecosystems

However, there is considerable debate on the best way to address these problems. There are many initiatives, from local to global scales, aimed at protecting current ecosystems and their associated biodiversity. Those chosen for protection are often reference ecosystems, areas recognised as remnant natural areas, relatively intact and not degraded by human activities such as agriculture, logging, mining or development and defined as being communities of organisms able to act as a benchmark reference target (Durbecq et al. 2020). In the context of changes in fishery stock, Pauly (1995) described an effect he called ‘shifting baseline syndrome’ where continuous (deleterious) changes over time lead each new generation of researchers to unknowingly accept a further degraded or altered environment as a baseline state, the reference point for what is considered a healthy or natural environment, therefore gradually shifting the reference baseline of what is perceived as an acceptable state over time. The concept of shifting baseline therefore suggests that reference to historical, pre-human intervention environments should be considered in order to resist acceptance of creeping ecological degradation. Conservation efforts may therefore seek to create resilient ecosystems, able to withstand change and disturbance without shifting to an alternative state, continuing to function as if the disturbance had not occurred (Horwath and Kuzyakov 2017). An alternate argument is that some disturbance of a moderate frequency may actually maximize species richness, the ‘intermediate disturbance hypotheses, and thus acceptance of some change, in specific circumstances, may actually be beneficial to biodiversity, although potential benefits from disturbance are not universal to all systems (Mackey and Currie 2001).

Whilst choosing these reference ecosystems as conservation baselines is aimed at preventing further deterioration of those areas, it can be argued that attempting to maintain stasis in protected areas when there is rapid change in global systems is not a sufficient response and that especially the current climate emergency renders these conventional conservation targets obsolete (Gardner and Bullock 2021). Novel, anthropogenically triggered ecosystems are already arising due to abiotic and biotic change, some being hybrid systems which retain some pre-human intervention characteristics and novel elements, others where larger scale change has resulted in novel systems, with different species, interactions, and functions to those that existed historically (Hobbs, Higgs, and Harris 2009). Recognition of these novel systems requires significant changes in conservation and restoration ideology, away from the traditional reference ecosystems (Hobbs, Higgs, and Harris 2009). Additionally, conservation efforts may be insufficient if they do not consider the importance of biodiversity in maintaining human civilisation, and this may need to include novel ecosystems, otherwise conservation efforts may be based on poorly formulated ideas of how to effect sufficient transformative change to achieve their ends (Gardner and Bullock 2021). These concerns have led instead to the concept of survival ecology, one which recognises the inevitability of change, but promotes the safeguarding of ecological systems, often novel ecosystems, where humans and other species can thrive in the future, rather than trying to recreate and maintain historic ecological norms (Gardner and Bullock 2021). With these differing arguments on how to evaluate the most effective ways to achieve ecological protection and thus how to promote biodiversity, many differing solutions, operating at different scales, are posited.

1.3 Natural climate solutions

Natural climate solutions are being suggested as climate mitigation by protecting and enhancing carbon capture and storage in plants, soils, and ecosystems (Schmitz et al. 2023; Seddon et al. 2021). The Intergovernmental Panel on Climate Change (IPCC) states that to meet the Paris Agreement target of limiting global warming to +1.5C there must be net zero carbon dioxide emissions by 2050 (IPCC 2022). The 2021 United Nations Environment Protection report (UNEP) states that nature-based solutions, the protection, restoration, and sustainable management of natural carbon sinks, can play a crucial role in climate mitigation, with a possible 24% of emissions removal being provided by efficient management of grassland for long-term carbon storage (Lera Miles and Sengupta 2022).

The IUCN Global Standard expects all nature-based solutions to have a net positive impact on biodiversity (Seddon et al. 2021). Whilst forests have obvious above ground biomass carbon storage, the below ground carbon storage potential of grasslands is more easily overlooked (Kristensen et al. 2022). Underground mineral-associated carbon stocks under grazed grasslands may be less vulnerable to perturbations than forests with higher above ground carbon storage, and thus support longer-term carbon storage persistence (Kristensen et al. 2022). Recognition that soils are the largest actively-cycling terrestrial carbon pool indicates a shift in thinking away from short-term carbon sequestration towards inclusion of whole ecosystem carbon persistence, and thus of the effects of large herbivores upon long-term carbon cycling and storage (Kristensen et al. 2022). Inclusion of these longer-term effects may have a more positive and synergistic impact on land management to protect biodiversity and climate mitigation (Kristensen et al. 2022).

Properly planned and managed, these solutions can also protect habitats and landscapes and animal species biodiversity, the animals themselves having a functional role in controlling the carbon cycle, enhancing natural carbon capture and storage (Schmitz et al. 2023; Seddon et al. 2021). It is estimated that natural biogeochemical processes within terrestrial and marine ecosystems already remove up to 50% of all human-caused CO_2 emissions and that appropriate protective management of existing ecosystems could reduce emissions from them by 4 $GtCO_2$ /yr, and further, that restorative management could increase their annual carbon storage capacity by 5–6 $GtCO_2$ (Schmitz et al. 2023). Well planned, the restoration schemes would simultaneously benefit biodiversity and fully utilise the recognised carbon storage function attributable to the megafauna that have diverse functional roles within those ecosystems (Schmitz et al. 2023; Malhi et al. 2022).

Since megafauna are so important for the functioning of biological communities, the structure of current wild ecosystems is strongly deviated from the longer-term evolutionary norm (Davoli et al. 2023). Megaherbivores can contribute to the capacity of ecosystems to adapt to climate change by enhancing the complexity of trophic webs, increasing habitat heterogeneity, and increasing resilience to abrupt ecosystem change, as well as through microclimate modification (Malhi et al. 2022). In the absence of megaherbivores, many current ecosystems exhibit decreased functional heterogeneity and decreased fluxes of biogeochemical compounds across the trophic networks when compared to pre-megafaunal mass extinctions (Davoli et al. 2023; Svenning 2020). To restore this ecosystem functioning, considerable upscaling of the current policies of megafauna community restoration would be required to facilitate the adaptation potential of ecosystems (Bergman et al. 2023; Davoli et al. 2023; Malhi et al. 2022).

1.4 Trophic rewilding with mega-herbivores

Trophic rewilding seeks to rehabilitate ecosystems by repopulating them with megaherbivores, thus re-establishing top-down interactions, and there are increasing examples from studies showing the positive effects on vegetative and faunal biodiversity of these introductions (Deli, Néstor, and Jens-Christian 2023; Bonavent et al. 2023; Ratajczak et al. 2022; Dvorský et al. 2022; Konvicka et al. 2021; Vodičková et al. 2019; Guyton et al. 2020; Hagstrup et al. 2020; Köhler, Hiller, and Tischew 2016). The diversity of below-ground is also reliant on top-down regulation. A study into the impact of the removal of large herbivores from long-term pasture found decreased homogenization for soil microbes and fauna, which are important for ecosystem functioning, and decreased carbon storage, after cessation of grazing (Schrama et al. 2023). Naturalistic grazing by large herbivores is therefore increasingly used as a way of managing habitats to restore and enhance biodiversity, aiming to create self-sustaining functional environments (Dvorský et al. 2022). Horses once had an extensive global range, and currently, despite severe range contraction since the Late Pleistocene, are still found in free-living populations worldwide acting as a functionally important grazer (Naundrup and Svenning 2015). Species distribution models that link climate and incorporate past and present distributions of free-living horses have identified areas suitable for rewilding them (Naundrup and Svenning 2015). These models, combined with habitat mapping, revealed that large areas would be suitable for rewilding with horses within their former range, including up to 1.5 million hectares within Europe (Naundrup and Svenning 2015).

1.5 The role of Exmoors in rewilding

Horses are already being extensively used in rewilding projects, with Rewilding Europe supporting free-living horses at eleven sites, covering 6100 hectares across Europe, with 400 horses (2019 figure) recorded in their European Wildlife Biobank (Rewilding_Europe 2024). Exmoor ponies are widely used to fulfil this functional role of horses in rewilding/ ecological restoration projects in the UK and Europe (Deli, Néstor, and Jens-Christian 2023; Bonavent et al. 2023; Dvorský et al. 2022; Tree 2023; Konvicka et al. 2021; Vodičková et al. 2019; Hagstrup et al. 2020; Tree 2019; Köhler, Hiller, and Tischew 2016). Investigation of the effect of Exmoor ponies' grazing on plant communities would inform their value in these projects and potentially secure them a role as eco-engineers, which would help to improve their conservation value into the future. Quantification of the time-scale of the impact of their grazing, by comparison of short- and long-term grazed plots, on the relative abundance and biodiversity of plant species, particularly of flowering plants, would offer insights informing their use as eco-engineers, particularly on sites where conservation of these plant species is prioritised. Comparison of these grazing effects between sites grazed by Exmoor ponies and domestic cattle and sheep would inform the relative value of these different herbivores, particularly on flowering plant-species targeted conservation.

It is recognised that the supply of suitable herbivores for restoration/ rewilding is sometimes limited, particularly if those animals are to be sourced from a rare breed population (https://rewildingeurope.com/news/banking-on-wildlife/). Rewilding Europe's European Wildlife Bank (EWB) was established to facilitate the breeding and exchange of herbivores between rewilding areas. Although not yet included in the EWB, Exmoor ponies are recognised as a 'priority' rare breed by the Rare Breeds Survival Trust (RBST), an oversight organisation for non-zoo bred, rare breed animal populations in the UK (https://www.rbst.org.uk/watchlist-overview). Exmoors underwent a genetic bottleneck in the 1940s when <50 individuals survived the Second World War and since then breeders have been working to preserve and increase the population (Baker 2008). There have been claims that Exmoors are likely to be closely related to the ancient wild horse found in NW Europe after the last Ice Age (Baker 2008; Hovens 2014; Dent 1962), based on: 1) their external phenotype, closely resembling that of the primitive equids depicted in cave paintings (BernÁldez-Sánchez and García-Viñas 2019); 2) their anatomical and morphological features, resembling ancient remains (Speed and Speed 1977; Hovens 2014); 3) their apparent retention of 'primitive' characteristics (Goodwin, Levine, and

McGreevy 2008; Ellegren 2002); 4) written evidence of their distinct characteristics, resulting in their being pure-bred in a discrete population over a long period at a time when many breeds were being deliberately cross-bred to ‘improve’ them (Sydney 1893; Hayes 1897); and 5) their adaptation to survive in harsh natural environments (Baker 2008; Hovens 2014; Speed and Speed 1977). Schafer (1981) hypothesises that modern horses are the descendants of four types of primitive equine and that the Exmoor pony closely resembles the primitive type he calls ‘the universal pony’. As a rare breed, believed to retain ancient characteristics, and since most Exmoors are bred in free-living herds under continual environmental selection pressure, they are of special conservation interest.

1.6 The origins of horse breeds

Before the increasingly widespread use of molecular methods to investigate the ancestry of domestic horse breeds, studies grouping the breeds into morphologically similar types and linking of these types according to their observed adaptive traits to their supposed areas of origin were frequently posited. Morphological studies into the origins of the domestic horse from the 19th and early 20th centauries indicate a widespread acceptance of the polyphyletic ancestry of modern domestic horses, often grouping them into ancestral lineages described as: cold-blooded; heavy horses of draught type perceived as coarse, sluggish, or phlegmatic in nature; or hot-blooded, finer boned, fierier natured light riding horses (Sydney 1893; Hayes 1897). Phrases such as ‘an ounce of quality is worth an inch of bone’ (Sydney 1893; Hayes 1897) when describing the breeding and selection of hunters and riding horses were common in the reference books of the time (Sydney 1893; Hayes 1897). The supposed superiority and refinement of the hot-blooded horses, usually of Arab type and of Middle eastern origin was so widely accepted during the 19th century that many native European breeds were crossed with usually imported Arabian or Barb stallions with the object of ‘improving’ them (Society ; Sydney 1893; Dent 1987). Exactly what these ‘improvements’ achieved and whether their effects upon a native breed was long-lasting is variable and open to debate. Lady Wentworth, a tremendous advocate of the crossing of British native ponies, widely advocated the use of Arab stallions on native mares and the influence of those crossings left its mark on the morphology of the offspring and in the Stud Book records, with many Welsh, New Forest and Connemara ponies having acknowledged Arabian and other outside influences from that period (Society ; Sydney 1893; Dent 1987). Appendix Table A1 is a summary of the references to the cross-breeding of the British native pony breeds, and shows that Exmoors seem to have largely escaped these ‘improvement’ efforts. Baker (2008) reports on the 19th centaury story of ‘Katerfelto’, a grey Arabian stallion whose semi-legendary exploits led to his being released on Exmoor and supposedly influencing the breeding of the wild ponies there. Baker (2008) extensively researches these claims and ultimately dismisses them as a local legend rather than a genuine influence on the Exmoor breed as an Arab stallion, lacking the hardiness and survival adaptations of the native Exmoor ponies, would certainly not have fared well on the moors in winter. Since ‘Katerfelto’ was described as a grey, and grey colouration is dominant in equines (Sponenberg 2000) it seems likely that any cross breeding would be easily detected by colour anomalies in a wild population (such as Exmoors) that is usually exclusively brown. More recently, the creation of what were originally meant to be dual purpose, draught and ridden breeds, by the deliberate crossing of cold and hot-blood breeds has created a separate category, the warm-bloods. There are now many European ‘sports horse’ breeds that are classified as warm-blooded, acknowledging their breeds’ origins for example, Cleveland Bay, Hanoverians, Trakehners and others) (Dell et al. 2020).

During the planning of the German State warm-blood sports horse breeding program (https://www.saechsische-gestuetsverwaltung.de/en/stud/the-german-state-studs/) there was considerable interest in the origins of modern equines and how their differing traits could be combined in breeding programs to produce horses with the capacity for high performance in different sports. Following extensive review of the theories of the origin of domestic horses Schafer (1981) described the ancestry of modern horse breeds as having their origins in two ancient groups according to their morphology. One group, which he described as the Northern Group Horses, was further divided into the Primeval Pony (Type 1) and the Ancestral Cold-Blood (Type 2); and a separate group which he called the Southern Group horses, was again divided into the Steppe Horses (Type 3) and the Proto-Arab Horses (Type 4). Interestingly, both types in the Southern group would have been described as hot-blooded, while he differentiates the Northern Group into the Ancestral Cold-Blood, and the Primeval Pony, which appears to escape classification as either hot or cold blooded but is distinctly different from the rest. Schafer (1981) showed a particular interest in Exmoor ponies. Probably influenced by the work of Ewart (1909) on the early origins of equines in Western Europe and by the morphological studies on Exmoor ponies by Speed and Speed (1977) at the University of Edinburgh, he made a special study of both their morphology and behaviour (Schafer 1981, 1975), where he describes them as retaining primitive morphological and behavioural traits which he suggests supports claims for their relatively direct and undiluted descent from the early Primeval Pony which colonised much of North Western Europe after the last Ice Age.

Unsurprisingly, whilst molecular studies find that many modern breeds do not show distinct clustering when their haplotypes are mapped, indicating the often-acknowledged admixture in their ancestry, the biogeographic and morphological-based groupings do sometimes show alignment in the findings of these molecular studies. The British and Northern European breeds, those which would be assigned to the Primeval Pony ancestral group (Type 1), are breeds which are shown to cluster together in several mtDNA studies (Jansen et al. 2002; Cieslak et al. 2010; Achilli et al. 2012; Castaneda et al. 2019; Royo et al. 2005). Since Exmoor ponies are core within this group and are likely to have contributed to the creation of some of these breeds, this is not an unexpected result (Appendix 1, Table A1.1).

1.7 The future of the Exmoor pony population

Despite having successfully grown the Exmoor pony population from the post WW2 bottleneck of <50 individuals, Exmoor ponies are still a rare breed (https://www.rbst.org.uk/watchlist-overview). The Exmoor Pony Society (EPS) website states that in November 2022 there were 3893 living pure-bred UK based Exmoor ponies registered in the Stud Book (94 licensed stallions, 2137 females, 1424 geldings and 238 other males (https://exmoorponysociety.org.uk/pony-faqs/). This remarkable numerical growth is attributable to the tremendous efforts of the EPS and individual breeders to save the breed from extinction. However, the overall total number of ponies is not a wholly accurate guide to the security of the population. Of the male population, only the sub-set of 94 licenced stallions are approved for breeding use, and many of those are not actively used as sires. Investigation of the actual effective breeding population and of the current representation of founder lines is therefore important to ensure the security of the breed in the future. Integration of pedigree and molecular information about the extant population, its genetic diversity, inbreeding and genetic load of deleterious alleles could help to inform conservation breeding decisions and thus contribute to the security of the Exmoor population into the future.

To have a secure future a breed must also have a purpose. A recent article in The Guardian newspaper highlighted an example of this in the Garrano pony, a rare native Portuguese pony (Jones 2024). Considered part of the Portuguese National heritage, most Garranos are kept as free-living herds in the Peneda Gerês National Park mountains (Santos and Ferreira 2012). Jones (2024) states that since the increase of mechanisation of agriculture in the region the Garrano's working role has become obsolete and the population has decreased to <3000 individuals. A 2005 study using 13 microsatellite markers showed that of the 277 sampled foals born in 1998, there were eight sub-populations corresponding to eight northern Portuguese geographic regions (Morais et al. 2005). Another mtDNA study showed them to fall into the same Northern European pony haplotype grouping as the Exmoor pony, rather than clustering with the more Southern Iberian horse breeds, indicating that they might share the same Primeval Pony ancestral group origin (Royo et al. 2005). Santos and Ferreira (2012) indicated that Garranos moving from the mountain scrublands to the better resources of the lower grasslands, and that this was leading to biomass accumulation in scrublands, with an associated higher fire risk. Freitas and Álvares (2021) reports that the return of the Garranos to scrub areas, whilst valuable for biomass reduction, is problematic for the ponies as increased wolf numbers result in the loss of <70% of foals born in the wild. While some losses are acceptable, the development of natural carnivore/ herbivore relationships is part of the rewilding ethos, and losses are compensated for by the Portuguese Institution for the Conservation of Nature and Forests (ICNF); however, such high losses could jeopardise the future population. A compromise, leaving the Garranos in the scrublands and thereby allowing them to continue in their role of biomass removal eco-engineers, but introducing an additional 'sacrificial' population of sheep or goats to divert most wolf predation, has been suggested (Jones 2024).

Similarly, Exmoor ponies must have a purpose. The EPS website states that most Exmoors are kept in domestic situations where they are used for leisure riding and driving, although the majority of breeding ponies are still kept in free-living herds and thus are still exposed to environmental selection pressures (https://exmoorponysociety.org.uk/introduction-to-exmoor-ponies/). Exmoor ponies are also increasingly used to fulfil the functional role of horses in rewilding/ ecological restoration projects in the UK and Europe (Deli, Néstor, and Jens-Christian 2023; Bonavent et al. 2023; Dvorský et al. 2022; Tree 2019; Tree 2023; Konvicka et al. 2021; Vodičková et al. 2019; Hagstrup et al. 2020; Köhler, Hiller, and Tischew 2016). These free-living herds, if appropriately managed, could be used to breed Exmoors suitable to supply the increasing demand for them as eco-engineers, whilst simultaneously protecting the diversity of genetic resources within the breed. However, this would depend on their retaining the traits that make them 'fit for purpose', that is, able to thrive as independent of human intervention as possible. Welfare considerations, such as fitness under minimal management, are therefore very important. Resource dependent seasonal changes in body condition are observed in populations of free-living equines (Debeffe et al. 2016; Jenkins et al. 2020; Putman et al. 1987). Their suitability for use in these minimal management situations depends on their ability to thrive in harsh environmental conditions, and especially on their ability to maintain their body condition adequately over-winter or during other periods of resource limitation. An additional challenge to body condition maintenance is that all equines carry a load of gastro-intestinal parasites acquired during grazing and there a negative correlation between body condition and parasite load has been found in the Sable Island feral horse population (Debeffe et al. 2016; Jenkins et al. 2020). Since many ecological restoration/ rewilding sites do not permit the routine use of anthelminthic treatments, investigation into the relationship between seasonality, gastro-intestinal parasite load and the maintenance of body condition in un-treated, free-living

Exmoor ponies would be of welfare interest in the context of their suitability for use in these situations.

Ripple et al. (2023) state that the climate crisis is now such that life on Earth is ‘under siege’ as a consequence of greenhouse gas emissions. They further state that what must now happen is the elimination of emissions from fossil fuels and land-use change, combined with increased carbon sequestration using nature-based climate solutions to address the combined climate and biodiversity crises. Nature-based solutions can contribute to reducing temperatures in the long term, long past the point at which net-zero emissions are achieved and peak warming has arrived, and thus could have a long-term effect on planetary cooling in the second half of this century (Girardin et al. 2021). Ecosystem restoration is recognised as central to conserving biodiversity and climate stabilisation, so that 47 countries have committed to have 150 and 350 million hectares of degraded lands under restoration by 2020 and 2030, respectively, as pledged under the Paris Climate Agreement (Strassburg et al. 2019; Strassburg et al. 2020). Efficient restoration of 15% priority areas might avoid 60% of expected extinctions, while simultaneously sequestering 30% of the total CO2 increase in the atmosphere (Smith et al. 2022; Hoffmann 2022; Strassburg et al. 2019; Strassburg et al. 2020). However, effective management in the face of these changes requires understanding of how these co-occurring threats affect species and ecosystems (Geary et al. 2023). Conservation has frequently aimed to prevent further degradation of protected areas but under rapid environmental and climate change this may be inadequate to protect vulnerable ecosystems and species (Hilderbrand, Watts, and Randle 2005). Restoration of ecosystems that have evolved over long periods is not a short-term process (Hilderbrand, Watts, and Randle 2005). The creation of novel ecosystems, different in composition and/or function from past systems is an almost inevitable consequence of changing species distributions and environmental alteration through climate and land use change, and can result in appropriate, locally adapted functional diverse ecosystems (Smith et al. 2022; Hobbs, Higgs, and Harris 2009). Rewilding may be part of ecological restoration or may create novel ecosystems, but if these function in ways towards a positive outcome for both the biodiversity and climate mitigation, it will be important to understand their formation, dynamics and functioning (Kerr et al. 2023).

1.8 Aims

This study investigated the role of Exmoor ponies in ecological restoration and rewilding, and their possible influence on the creation of novel ecosystems through their grazing interactions. It investigated their future survival potential by addressing their ability to maintain healthy body condition under minimal management regimes, and the effect of past breeding management their future population viability.

The aims of this PhD are therefore:

- To investigate the impact of Exmoor pony grazing on vegetation biodiversity and flowering plant abundance, using manipulation experiments, to explore long-term diversity outcomes, short-term inclusion and exclusion effects and the comparative impacts of Exmoor ponies and domestic cattle and sheep.

- To investigate impact of parasite load and seasonality on body condition in a herd of free-living Exmoor ponies to inform their suitability for minimal intervention grazing projects.

- To combine pedigree and molecular data to improve informed conservation breeding management of Exmoor ponies to improve the future security of the population.

1.9 Chapter objectives

The first stage of my thesis was to investigate the effect of Exmoor pony grazing. There was considerable anecdotal evidence suggesting that their grazing habits had a positive impact on the biodiversity of sites where they grazed, therefore in **Chapter 2**, I conducted a study to investigate whether this effect could be quantified. Using existing enclosures, I undertook quadrat sampling methods, recording vegetation species presence and abundance at the same locations and vegetative phenological stage for three years. I was able to collect data from quadrats where Exmoor ponies were newly introduced to previously un-grazed areas; Exmoor ponies were excluded from an area previously grazed by them; Exmoor ponies had been exclusively continuously grazing for > 30 years; commercial cattle and sheep had been continuously grazing for > 30 years; and an un-grazed area. This data was then analysed to investigate the effects of the differing grazing treatments.

Exmoor ponies are widely used for conservation grazing and ecological restoration/ rewilding projects. Many of these projects do not permit routine use of anthelminthic medications or even of seasonal supplementary feeding. In **Chapter 3,** I investigated the relative impacts of seasonality and parasite load on the body condition of Exmoor ponies. These factors are relevant to the welfare of Exmoor ponies kept in minimal husbandry situations, and in the selection of suitable stock for restoration/ rewilding projects.

Exmoor ponies are a rare breed which passed through a genetic bottleneck of <50 individuals in the 1940s. Thanks to the dedication of breeders the breed has seen a rise in numbers to a total population of >2000 ponies. In **Chapter 4,** I investigated the impact of

the genetic bottleneck and of past breeding management on the current population. DNA was extracted from hair samples collected from a broad cross-section of the extant UK Exmoor pony population. mtDNA and whole genome sequence data obtained from these samples was then used, in combination with pedigree data, to verify founder matrilines, and investigate inbreeding, within the Exmoors, and in comparison with other horse breeds, and genetic load in the Exmoor population.

Finally, in **Chapter 5,** I discuss the implications of the results of this study in the context of the conservation breeding management of the population, and the potential future role for Exmoor ponies in ecological restoration and rewilding.

Chapter 2: Effects of grazing manipulations, using Exmoor ponies, on biodiversity and flowering plant abundance

2.1 Abstract

There is global recognition of the loss of biodiversity and of the threats posed by climate change. Grasslands provide many crucial ecosystem services and, when properly managed can be biodiverse environments, act as carbon sinks, and have a potential role in climate mitigation. Semi-natural grasslands with a high abundance of flowering plants (forbes) have been_shown to be associated with carbon-rich soils and provide enriched habitats for pollinators. Grazing by appropriate herbivores can help to create these habitats. Rewilding and ecological restoration schemes sometimes use_mega-herbivores as eco-engineers to restore such ecosystem functions. However, which mega-herbivore is most appropriate is often not tested prior to the initiation of these rewilding studies. This study used exclusion, inclusion and unmanipulated grassland areas to test whether grazing the effects of different grazing treatments, including grazing by Exmoor ponies, ancient analogue mega-herbivores, and by commercial livestock had different effects on overall vegetative biodiversity and specifically on flowering plant (forbe) abundance. Over the span of three years, overall biodiversity was positively impacted, and it was found that grazing by Exmoor ponies increased the abundance of flowering plants when compared to the other treatments, including grazing by commercial cattle and sheep. Although initially results suggested that the removal of Exmoor ponies had negative impacts on grassland, contrarily a dynamic Bayesian model suggested that grazing by Exmoor ponies would have a persistent, positive effect on both overall and specifically flowering plant diversity. Further investigation might elucidate the possible longer term impacts of inclusion and exclusion of Exmoor ponies. The study results indicate the potential of Exmoor ponies as eco-engineers to improve the overall biodiversity and the abundance of flowering plants (forbes) on conservation sites and in rewilding projects.

2.2 Introduction

There is increasing recognition that there is a biodiversity crisis, exacerbated by climate change, pollution, and habitat fragmentation and loss, the severity of which has tremendous implications for the survival of life on earth. Worryingly, the negative effects of climate change are accumulating even more rapidly than previously reported (Tollefson 2022). If there is a rise in global temperature >1.5 ºC some environmental damage is likely to be irreversible. Tollefson (2022) argued that it is crucial to protect biodiversity and natural ecosystems as part of the potential mitigation of these damaging changes.

Species extinction rates are such that the Earth may be in the 6th Mass Extinction period in its 4.5-billion-year history. This accelerating decline in biodiversity is may significantly impact ecosystem services critical to human health and well-being; for example, a decline in pollinators could have substantial effects on agricultural production and food security, within less than a hundred years (Ceballos et al. 2015). This is a warning that action is needed now to mitigate the potentially drastic future impacts of the climate and biodiversity crisis.

Nature-based solutions to the biodiversity and climate change crises, – i.e., those based on increasing ecosystem resilience and adaptation focusing on carbon sequestration and management through reduced deforestation and reforestation, – are increasingly recognised by policymakers (Malhi et al. 2022), although controversy remains about their aims and effectiveness.

The importance of grasslands, temperate and savannah, as carbon sinks is often overlooked and afforestation of these areas, promoted as a carbon mitigation may, without careful planning, actually yield a perverse result. Dudley et al. (2020) expressed concerns about ignoring the importance of grassland as a long-term, sub-soil carbon sink; -for example, the 2021- 2030 UN Decade on Ecosystem Restoration, focuses primarily on re-afforestation instead of grassland regeneration, although this might undermine appropriate and effective carbon management strategies in some areas because grasslands could provide long-term sub-soil carbon sinks (Dudley et al. 2020). Kristensen et al. (2022) suggests that the pressure to plant trees as carbon sinks as part of climate mitigation strategies sometimes overlooks the long-term persistence of carbon in soils as mineral associated organic matter (MAOM), which is higher under grassland than under forest, the organic matter residence times in subsoils under grassland being nearly twice that of the persistence under woody habitats (~5400 years relative to ~2800 years) (Kristensen et al. 2022). In fact, grasslands store up to 10-30% of the global soil organic carbon (SOC) and are thus an important carbon sink (Chang et al. 2021). In a five-year study of changes in SOC storage under differing herbivore grazing assemblages the SOC was found to be highest in grasslands where the forb: graminoid ratio was higher than in graminoid dominated grasslands (Chang et al. 2021). The lowest SOC levels were associated with predominately sheep grazed areas, as sheep consumed a higher percentage of forbes than the other herbivore assemblages in the study.

Since climate change can also impact the SOC by changing plant carbon inputs and altering soil microbial activity, appropriate grazing management and biodiversity restoration to maximise SOC storage can act as a natural climate change mitigation in grasslands (Bai et al. 2022). Bai et al. (2022) states that achievable SOC sequestration potential in global grasslands is 2.3 to 7.3 billion tons of carbon dioxide equivalents per year (CO_2e year^{-1}) under biodiversity restoration schemes, and 148 to 699 megatons of CO_2e year^{-1} under improved grazing management, indicating the value of appropriate grassland management as a natural climate change mitigation solution.

The Paris Agreement, a legally binding international treaty on climate change made at the UN Climate Change Conference (COP21) in Paris, in 2015 agreed a limit to global warming of 1.5^0 C at the end of the centaury and net zero emissions by 2050 (Schmitz et al. 2023). To achieve the removal of 6.5GtCO_2 /year $^{-1}$ required to meet these targets, trophic rewilding on a much larger scale than at present, using herbivores as eco-engineers to assist in carbon storage, may be required (Schmitz et al. 2023). Pettorelli et al. (2018) suggest that integrated restoration and rewilding strategies may be the way forward to exploit the synergies of combatting biodiversity loss and climate mitigation (Pettorelli et al. 2018). Large herbivores are important as eco-engineers; therefore, the use of large, charismatic, rare, and endangered animals, an obvious focus for public engagement in conservation strategies as ambassador species, might also have an increasing role in ecological restoration and rewilding projects (Malhi et al. 2022).

The elimination of such megaherbivore influence is thought to be one the major factors leading to habitat changes at the end of the Pleistocene period (Owen-Smith 1987). Owen-Smith (1987) formulated the “keystone herbivore” hypothesis, which was extrapolated from observation of the grazing habits of rhinos and other extant African megaherbivores, proposing that their influence created spatially diverse vegetation patterns, open woodland, and grassland lawns, which in turn favoured the abundance of smaller herbivores dependent upon the diversity of the habitat for nutrition and shelter (Owen-Smith 1987). This idea has been supported in more recent studies; for example, African elephants have been found to reduce woody plant density from between 15-85% by grazing and trampling, thus favouring the creation of open woodland and grazing lawns (Malhi et al. 2016). This may indirectly provide a simultaneous benefit for tree species by nutrient cycling, reducing competition from other vegetation, and the effect of understorey removal on fire. A review by Cromsigt et al. (2018) emphasises the value of large wild animals rather than domestic livestock, especially non-ruminant animals with lower methane production, as especially valuable in these roles, as well as being of conservation interest in themselves. One example cited is the use of large herbivores in Siberia's 'Pleistocene Park', where the methane production of introduced herbivores is more than offset by the positive effect of their interactions with the vegetation slowing tundra thawing associated with methane release. Another is the increased grazing pressure of wildebeests in the Serengeti reducing scrub cover and thereby fire incidence and thus converting the area, previously a carbon source, to a carbon sink. These examples support the idea put forwards by Mahli (2022) that the observed sixfold decline in the geographical range and the abundance of megaherbivores post-Pleistocene could thus be a driver of the loss of global biodiversity (Malhi et al. 2022).

Malhi et al. (2016) describe how, dependent upon grazer density and other environmental factors, African elephants can reduce woody plant density from between 15-85% by grazing and trampling, thus favouring the creation of open woodland and grazing lawns. This may indirectly provide a simultaneous benefit for tree species by nutrient cycling, reducing competition from other vegetation, and the effect of understorey removal on fire. Cromsigt et al. (2018) emphasises the value of large wild animals rather than domestic livestock, especially non-ruminant animals with lower methane production as especially valuable in these roles, as well as being of conservation interest in themselves. One example cited is the use of large herbivores in Siberia's 'Pleistocene Park', where the methane production of introduced herbivores is more than offset by the positive effect of their interactions with the vegetation slowing tundra thawing associated with methane release. Another is the increased grazing pressure of wildebeests in the Serengeti reducing scrub cover and thereby fire incidence and thus converting the area, previously a carbon source, to a carbon sink (Probert et al. 2019).

It can be argued that before human impact, natural forest in Western Europe in the pre-Neolithic times, circa 7000 years ago, was a biodiverse, open-canopy, woodland pasture shaped by the numerous and diverse megaherbivores, and suggests that a return to this original-natural landscape should be a high conservation priority (Vera 2000). The Megaherbivore hypothesis suggests that grazing by large herbivores, particularly the keystone species, which vary according to the specific environment, are the major drivers of the development of vegetative communities. A synthetic analysis of beetle fossils from Britain pre- the Pleistocene extinctions found evidence for the presence of abundant and diverse megaherbivores associated with high diversity of vegetation before the Pleistocene megafauna extinctions (Sandom et al. 2014). This supports the hypothesis of high biodiversity in the presence of abundant Pleistocene megafauna.

Cromsigt et al. (2018) indicate that the ongoing rapid decline of megaherbivores is causing a restructuring of ecosystems that will negatively impact on human lives and suggest that the legacy effects of the megafaunal extinctions of the Pleistocene Era resulted in global consequences, including climate warming, large-scale vegetation shifts and reduced carbon sequestration. This implies that conservation and restoration of megafauna and the landscapes that they create may contribute to mitigation of current anthropogenic climate change. Therefore, the grazing of megaherbivores, especially of analogues to those in late-Pleistocene ecosystems, resembling extinct ancient rather than extant modern species, may restore trait combinations lost in the extant species, and thus drive landscape scale changes and restore ecosystem functions (Lundgren et al. 2020).

These reintroductions of megafauna, their management, and their effectiveness are the subject of considerable controversy and debate. Naundrup and Svenning (2015) and Bakker and Svenning (2018) assert that megafauna, particularly keystone species, can have huge impacts as ecosystem engineers and their loss can have a profoundly negative effect on ecosystems. They state that rewilding, the reintroduction of species or functional types with varying degrees of human intervention, to restore self-regulating, functional, biodiverse ecosystems is being increasingly implemented and that there is considerable scope for massive expansion of rewilding as tool for ecological restoration.

Rewilding is described as the management of ecological succession, with minimal human intervention, to restore natural ecosystem processes (Hagstrup et al. 2020). Nogués-Bravo et al. (2016) state that rewilding through the re-introduction of species, often megaherbivores, can stem the loss of biodiversity and ecosystem services. This is in accord with the hypothesis that it was the loss of these megafauna that led to past environmental changes analogous to those currently occurring (Cromsigt et al. 2018).

These ideas have been very influential in the rewilding movement, including in the UK, where Knepp Estate in Sussex is a flagship ecological restoration project that has raised public awareness of rewilding to promote ecological restoration, particularly through the publication of “Wilding” (Tree 2019) and “The Book of Wilding” (Tree 2023). These books highlight the changes to the landscape and to the species it supports during its transition from agricultural land to an environment shaped by the ecological engineering of introduced megaherbivores and offer guidance for the initiation of rewilding schemes on different scales and from different baselines. Such schemes vary from >1000-hectare schemes environments, where with megaherbivores, their populations population size naturally regulated by the carrying capacity of their habitats and the presence of carnivores, with little or no human intervention, through to <1 hectare or even garden garden-scale projects where human management must perform the regulatory role to support functioning ecosystems. Increasing public interest has led to pressure on policymakers to increase support for ecological restoration. For example, Trouwborst and Svenning (2022) highlight the legal obligation, under Article 8(f) of the European Convention on Biological Diversity, which requires restoring the diversity and densities of Europe's megafauna, which they have argued will increase ecological resilience to anthropogenic change. However, what has been lacking are manipulative studies to assess the impacts of different megaherbivores on ecosystem dynamics.

A review paper Malhi et al. (2022) highlights the complexity of ecosystem interactions. Untangling the impacts of vegetation-, animal-, ecosystem-, and climate-interactions and possible synergistic effects between them in manipulation studies of interventions into biodiversity, conservation and climate change mitigation is challenging. Kristensen et al. (2022) indicate that restoration initiatives aimed at providing such synergistic biodiversity and climate benefits must have a hypothesis-focused evaluation of effectiveness and clearly show the intervention contexts likely to offer positive outcomes. Further investigations may show that some interactions are negative or involve trade-offs, but others may be mutually beneficial. Recognising the role of ecological restoration in the enhancement of biodiversity, and its associated potential increase in resilience to rapid climate change, this study investigates the effects of different grazing treatments on the biodiversity of vegetation and abundance of flowering plants. In a meta-analysis of 109 studies looking at the multi-trophic effect of cattle grazing, Filazzola et al. (2020) found that livestock exclusion increased abundance of all species across all trophic levels except for detritivores. They noted that the number of years since livestock exclusion strongly influenced plant species abundance and that the effects of grazer exclusion was strongest in temperate regions. Numerous studies show that the effect of livestock on plant communities depends on the livestock type, management system, frequency, intensity and duration of grazing, and latitude, altitude, and climate ((McIntyre and Lavorel 2001), (Dorrough, Ash, and McIntyre 2004); (Hickman and Hartnett 2002); (Sjödin, Bengtsson, and Ekbom 2008); (O'Connor et al. 2010)) although most suggest that high grazing pressure by livestock, particularly in intensively grazed agricultural grasslands, is correlated with negative effects on plant community diversity.

Bonavent et al. (2023) suggest that historic declines in mega-herbivore grazers have led to a legacy effect of reduced forbe species abundance, creating the more prevalent modern graminoid dominated European semi-natural grassland. Bonavent, while stating that grazing is necessary to maintain high vegetative alpha diversity, overall species richness, and the functioning ecosystem services associated with this diversity, such as pollinator abundance, also highlights that increased species unicity, the increased abundance of locally unusual forbe species may be of equal or greater importance than maintenance of high vegetative alpha diversity or overall species richness.

A meta-analysis by Tälle et al. (2016) investigating the relative effectiveness of grazing and mowing in retaining phyto-diversity across 35 studies, focused on semi-natural grasslands, found that overall, there were small but specific advantages on phyto-diversity to grazing over mowing in most studies., which showed on semi-natural grasslands selective grazing had a less intrusive effect on the biodiversity present at sites. This was probably due to the selective nature of the grazers and their creation of more diverse habitats with greater vegetative structural complexity, with lawns and ‘rough’ (less heavily grazed) areas. These more complex habitats also tended to have a ‘knock on’ effect of supporting a greater variety of invertebrate and bird life. These conclusions are supported by Coverdale and Davies (2023) who state that vegetative structural complexity is always positively correlated with phyto-diversity. However, grazing might not be the only solution to increasing vegetative complexity. To investigate whether re-seeding could improve phyto-diversity and increase functional ecosystem services associated with high alpha diversity on graminoid dominated intensively grazed agricultural grassland in Europe, Freitag et al. (2023) re-seeded 73 sites to improve vegetative diversity. Although a 10-35% improvement in phyto-diversity was observed across the re-seeded sites, there was little improvement in ecosystem services, except nitrogen cycling, suggesting that this method would not be effective where the existing grassland vegetation had already saturated the available functional niches.

The selectiveness of grazing might also vary by particular species. It has been hypothesised that ancient-analogue megaherbivores may bring trait combinations beneficial to vegetative biodiversity, due to their robustness to withstand natural conditions without interventions and their selective grazing (Lundgren et al. 2020). For example, Exmoor ponies, an endangered breed, already widely used as ancient equines analogues in rewilding and restoration schemes due to their retention of primitive conformation, behavioural and survival traits ((Baker 2008); (Hovens 2014); (Tree 2019); (Hagstrup et al. 2020)). To investigate the effects of grazing by semi-feral, ancient analogue cattle (Galloways) and horses (Exmoor ponies) on alpha vegetative diversity and vegetative unicity, Bonavent et al. (2023) applied five grazing treatments to plots of semi-natural grassland in Denmark. Treatments included full exclusion of the mega-herbivores, seasonal grazing, seasonal mowing, and year-round grazing. They concluded that grazing, whether year-round or seasonal, had a more positive effect on both the alpha diversity and the unicity of the vegetation in the study. These results concur with the conclusions of Vodičková et al. (2019) and Konvicka et al. (2021) who both report increases in alpha diversity across multiple trophic levels on sites grazed exclusively by Exmoor ponies in the Czech Republic. However, all these studies included only results collected from areas where the treatments were either recently introduced or introduced for the purpose of the study. This would suggest that the habitats were potentially undergoing changes due to recent introduction treatments. None compared results from these introduction treatments with results from long-term treatments, for example areas where the treatment had been consistent for >30 years and so unlikely to be undergoing change due to recent variation in the treatment. Only Bonavent et al. (2023) included an exclusion treatment in their study. However, as their study included simultaneous grazing by both Exmoor ponies and Galloway cattle the effect of the different species (equine hind-gut digestors with upper and lower incisors and bovine ruminants with only lower incisors), whose grazing behaviours and preferences are different, could not be disentangled. None included a comparison of the grazing effects of modern commercial livestock with breeds used as ancient-analogue megaherbivores, hypothesised to bring trait combinations beneficial to vegetative biodiversity.

2.3 Aims

The aim of this study was to investigate the effect of different grazing manipulations, including the grazing by Exmoor ponies on (1) the overall biodiversity of plants; (2) specifically the abundance of flowering plants and (3) to model the long-term effects of the introduction or removal of Exmoor ponies on plant biodiversity and flowering plant abundance. Five grazing treatments were applied to eight separate areas (1) three unmanipulated areas grazed for >30 years solely by Exmoor ponies: (2) one exclusion treatment where ponies were removed from an area previously grazed for >30 years; (3) two areas where ponies were introduced to areas previously un-grazed by any stock for >10 years; (4) one unmanipulated area grazed solely by commercial cattle and sheep (livestock) for >30 years; and (5) one unmanipulated area un-grazed by any stock for >15years. This study therefore allowed comparison of long-term pony grazed and livestock grazed areas where the grazer: vegetation relationship had reached an equilibrium with exclusion and inclusion areas likely to undergo a shift in the relationship due to manipulation of grazing treatments and an un-grazed area. This study therefore investigated any differences in effects on biodiversity and flowering plant abundance of the long-term grazing of different herbivores, and on the introduction or exclusion of Exmoor ponies, or the absence of grazers on overall biodiversity and on the abundance of subsets of vegetation under different grazing treatments. The results were then used to create a predictive model of the impact of the ponies' grazing. A limitation of the study was that previously fenced enclosures were used so that, although chosen to be as similar as possible, areas under comparison could not be perfectly matched for size and environmental conditions.

2.4 Methods and Analysis

2.5 Location of study

This study was conducted in the north-west Highlands of Scotland. The two areas chosen for the study, Scoraig peninsula and Durnamuck, Dundonnell, both in Ross-shire, Highland Region (Figure 1.1). Their geographical proximity meant that it was expected that they would ~~be~~ similar in terms of habitats, exposure, geology, soil type, weather, and climate. Durnamuck is on the south side of Little Loch Broom, having a north-facing aspect approximately 1 mile across the sea loch facing the Scoraig. Whilst both have a history of subsistence crofting agriculture since the Clearances of the Highlands in the 18th century, recently the land use has differed both in the types of livestock kept and the intensity of land use so that, whilst abiotically similar, the chosen areas allow investigation of differences in history of grazing.

The study area at Durnamuck is an area of apportioned hill ground, meaning that it was formerly common land extensively and sporadically grazed by sheep and deer, with sheep being introduced during the summer in some years, and deer incursions, although minimised by fencing and hunting, occurring in the late winter or early spring. Enclosed in the 1990s, it was grazed by sheep and cattle until the early 2000s when the crofter effectively retired, and the land was un-grazed except by occasional incursion by red deer. The study area included two adjacent areas of approximately 20 hectares each, with the same: underlying rock. Torridonian sandstone; soil conditions; elevation; aspect; drainage; and exposure to weather. They also had apparently similar vegetation cover, thus offering two edaphically similar areas to which the effects of grazing treatments on the vegetation could be investigated, whilst minimising other environmental influences.

The study areas on Scoraig peninsula share the same underlying rock as Durnamuck, Torridonian sandstone. On the south side of the peninsula two areas on the same croft, separated by a few hundred meters, were investigated during the study. Both had been enclosed croft land used for grazing cattle and sheep since the 18th Century, with similar elevation, aspect, slope, drainage, and soil. Neither area had been grazed previously by Exmoor ponies. One area had been consistently in use for livestock grazing; the other had been un-grazed, except for occasional incursion by roe deer, for approximately 15 years due to difficulties in access.

On the north side of the peninsula, four areas were investigated during the study, involving two sets of adjacent fields separated by a few hundred meters on the same croft.

These had been croft land used for grazing cattle and sheep since the 18th Century, sharing similar elevation, aspect, slope, drainage, and soil. These areas also shared a recent grazing history different to the other areas in the study, having been grazed exclusively by Exmoor ponies for > 30 years. These study areas are abiotically similar to those under investigation elsewhere on Scoraig except that they have a north-facing aspect.

The choice of these study areas allowed comparison of differing grazing treatment impacts on the vegetation with minimal other environmental variation between them. The areas shared similar historic grazing histories, with variation in recent grazing enabling comparison of its impact upon them. Maps of the treatment areas at the study sites are in Appendix 1.

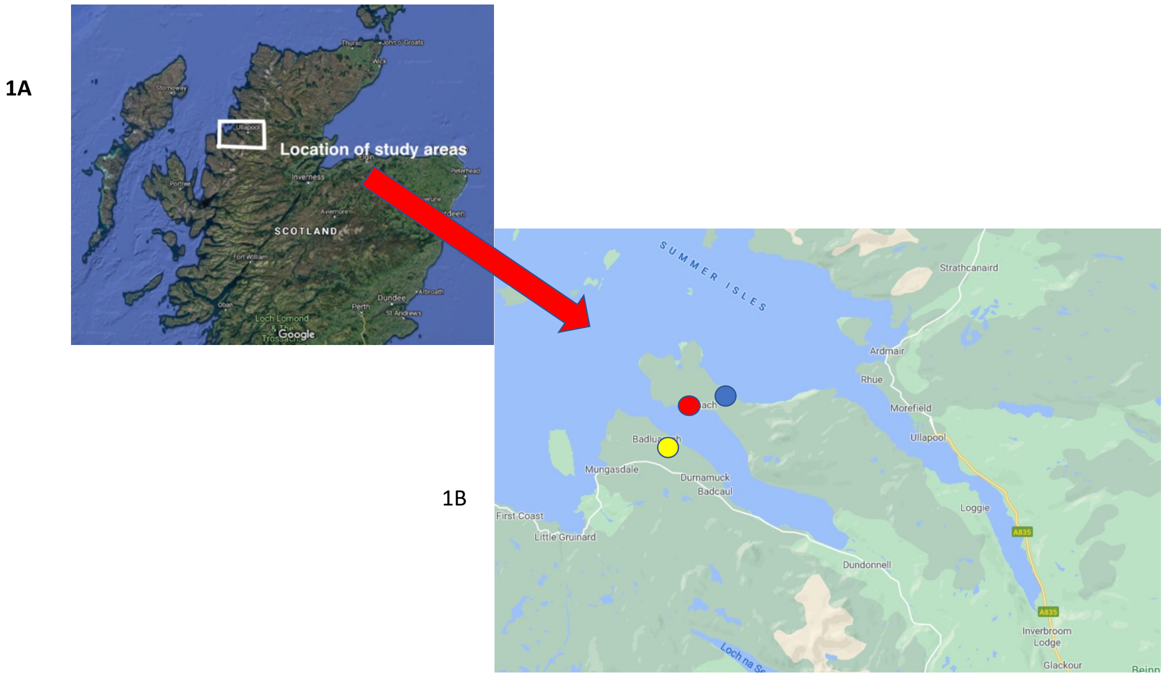


Figure 1.1: Location of the study. 1A The location of the study areas within the Highlands of Scotland and 1B The location of the study areas within Dundonnell. The yellow dot indicates the location of the Durnamuck areas. The red dot indicates the areas on the south of the Scoraig peninsula. The blue dot indicates the areas on the north of the Scoraig peninsula.

2.6 Grazing treatments applied during the study

During the study, five treatments were applied to investigate their impacts on the vegetation in the three study area's locations (Appendix 2: Figures A2.1-A2.3).

The treatments were: (A) pony grazing– 3 areas grazed exclusively by ponies for >30 years; (B) pony inclusion – ponies were introduced to 2 areas previously un-grazed by any stock; (C) pony exclusion – ponies were excluded from areas previously grazed exclusively by them for >30 years during the study; (D) livestock treatment – an area consistently grazed by commercial cattle and sheep (modern breeds, not ancient analogue breeds) but not ponies for >30 years; and (E) un-grazed – an area never grazed by ponies and un-grazed by livestock for >15 years. Due to differences in available habitat and history of grazing, different treatments were applied in each area (Table 1), with different areas of treatments at Durnamuck (Appendix 2: Figure A2.1); Scoraig Peninsula north (Achmore croft; Appendix 2: Figure A2.2) and Scoraig Peninsula south (Scoraig Croft; Appendix 2: Figure A2.3). Four ponies were introduced to the Durnamuck inclusion treatment area in mid-September 2019. Two ponies were introduced to the inclusion treatment area on Scoraig in August 2020. _The differing numbers of ponies was due to the smaller area on Scoraig being unable to support more ponies. Ponies were removed from the exclusion plot at Achmore in May 2020. There were 3 pony grazed plots at this site because they were contained in separate, fenced paddocks, as well as the one exclusion plot.

Table 1.1: Grazing treatments. The different grazing treatments applied to investigate their effects on the vegetation with otherwise minimal environmental variations at Durnamuck and Scoraig, Highland, Scotland.

Study area	**Durnamuck**	**Scoraig**	
		North	**South**
Treatment	(A) **Inclusion -** ponies introduced to the area during the study. Area previously un-grazed for approximately 15 years	(A) **Grazed-** 3 areas grazed continuously and exclusively by ponies for >30 years	(B) **Inclusion –** ponies introduced to the area during the study to areas previously un-grazed for approximately 15 years
	(B) **Un- grazed -** Area un-grazed for approximately 15 years	(C) **Exclusion –** ponies removed from an area previously grazed for >30 years	(D **Livestock-** area grazed exclusively by commercial (not ancient breed) cattle and sheep for >30 years

2.7 Vegetation surveys

Quadrat sampling

On the Scoraig peninsula plots, quadrats were randomly positioned within a subset of locations within each site. Locations to sample within the plots were chosen to give a range of possible biotic (grazing) and edaphic (especially drainage and past soil management) factors, giving a total of 30 quadrats. Orientation was noted in degrees using a handheld Global Positioning System (GPS) to facilitate identification of the positions of the quadrats for resurvey in the future. Each was marked at the SW corner with a metal peg driven well into the ground. The sampling periods were: 30/05/20-15/06/20, 15/06/21-03/07/21 and 28/06/22-04/07/22. The aim was to sample the plants when at the same stage of their phenology each season, where possible. The sampling method used was the same at all plots, every year.

At Durnamuck, the sampling periods were 10/06/20-07/07/20 and 10/06/21-22/06/21. Quadrats were randomly positioned, three for each of the two treatment plots within the site, approximately 3m apart. Nine quadrats were sampled from each of the treatments, the inclusion area grazed by ponies and the adjacent exclusion area, giving a total of 18 quadrats sampled overall. Each was marked at the SW corner with a small (3-4 cm diameter) square wooden post with an ID number painted onto the top driven well into the ground. Orientation was noted in degrees using a smartphone compass. GPS co-ordinates were used to identify the positions of the quadrats for future resurvey. Sampling was delayed at Durnamuck due to Covid-19 restrictions. Unfortunately, it was not possible to sample at Durnamuck in 2022 because of a change in land ownership.

Obtaining ground cover scores

The first phase of the study aimed to establish baseline data on the vegetation cover of the sites. This was done using the protocol from the Climex Handbook of Standardised Experimental Methods (Jaroszynska 2019) for establishing plant community composition. Initial surveying was carried out between 30/05/20 and 08/06/20 and during June 2021 using 50cm^2 metal quadrats divided into 5cm^2 squares, 100 squares per quadrat. The presence of a species, rooting within a square, was recorded by progressive scoring out of a possible total of 100 for each species per quadrat. Foliage was moved to score plants hidden below to give the vegetation score. The presence of rocks, manure, and bare ground and litter (dead vegetation) within the sample quadrats was recorded by the same progressive scoring as for vegetation presence. The score for each category of ground cover, vegetation or other, was recorded and was used as the coverage abundance for each category of ground cover in the analysis. Plants were identified to as low a classification as possible by their vegetative morphology if they were not in flower at sampling time. Sampling was then repeated in 2021 on Scoraig and at Durnamuck, and in 2022 on Scoraig using the same sample quadrats to record any changes observed in vegetative abundance and diversity.

2.8 Statistical analyses

Explorative Principal Component Analysis

An explorative Principal Component Analysis (PCA) was run using the *vegan* package in R Studio (R Core Team 2013). To facilitate this analysis, cover values of individual plant species per site and year were aggregated into their corresponding functional types (i.e., bryophytes, graminoids, heather, herb, lichen, and trees). Using this information, differences in treatments were established as driven by abundance of functional types of vegetation to identify differences between treatments and changes across different years between the treatments.

Static Linear Models: Shannon's diversity indices

To identify the direct effect of Exmoor ponies on vegetation diversity, general linear models were constructed. Within these models, overall vegetation diversity, as measured through Shannon Diversity Index per site and treatment, was related to the additive effects of time since Exmoor pony removal and introduction to the site as measured in years. To analyse the ground cover scores the results of the vegetation scores obtained from the quadrats were visualised using *ggplot* in R.

The Shannon Diversity Index for each quadrat was calculated in R. The Shannon Index was chosen as it indicates both the species richness (the number of species) and evenness (the relative abundance of each species) and is measured on a 0 – 1 scale. Higher scores indicate higher levels of diversity. However, high variation of species or uniformity of individual distribution among species may result in a higher Shannon Index.

The Shannon formula: $H = -\sum[(p_i) * \log(p_i)]$ where H = Shannon index, p_i = proportion of individuals of i-th species in a whole community: $p_i = n / N$, n = number of individuals of a species, N = number of individuals in the community, log = natural log. The proportion of each species, p_i, was obtained by dividing the number of individuals of a species by the total number of individuals in the community. For each species, the proportion was multiplied by the logarithm of the proportion. The Shannon Diversity Index is then obtained by multiplying the sum of all the individual species calculations by -1. These calculations were performed in R.

The results from the quadrats sampled in each of the five treatments in the study: (A) pony grazed– areas grazed exclusively by ponies for >30 years; (B) pony introduction

– ponies were introduced to an area previously un-grazed by any stock; (C) pony exclusion –ponies were excluded from an area previously grazed exclusively by them for >30 years; (D) livestock grazed– an area consistently grazed exclusively by cattle and sheep, but not ponies, for >30 years; and (E) un-grazed – an area at Durnamuck un-grazed by either ponies or livestock for >15 years were modelled. “Livestock. grazed” in this study refers to grazing exclusively by cattle and sheep, while “pony grazed treatment” refers to grazing exclusively by Exmoor ponies. Individual plant species were additionally allocated into five groups to allow comparison of the relative abundance of each group under each treatment: grasses, sedges, rushes, flowering plants, and mosses (bryophytes). Although lichens and tree seedlings were also found in some quadrats these were either not grazed by herbivores (lichens) or so scarce (<5 tree seedlings overall) that they were not included in the analysis.

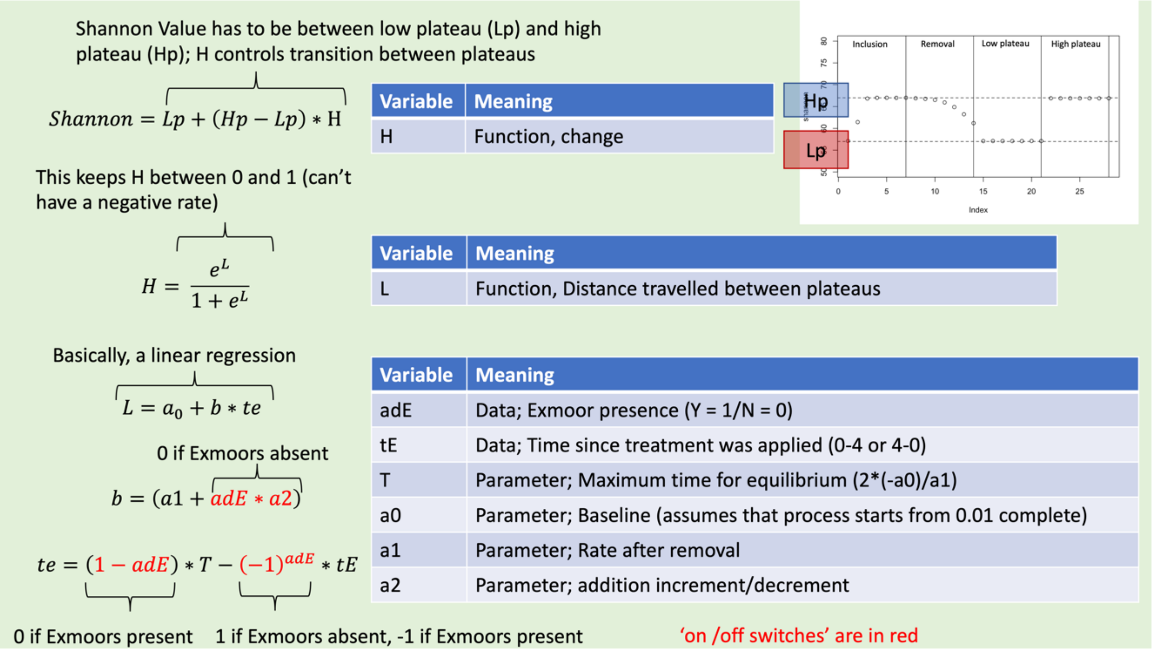
Using R Markdown, (JJ Allaire, Yihui Xie, Jonathan McPherson, Javier Luraschi, Kevin Ushey, Aron Atkins, Hadley Wickham, Joe Cheng, Winston Chang and Richard Iannone (2021) rmarkdown: Dynamic #> Documents for R) a full general linear model (glm) was created with Shannon’s diversity index as response variable and treatment and year and their interactions as explanatory variables, then repeated without the interaction. logLik was used to measure model fit, and to compare the two models. This likelihood ratio test was used to determine whether the interaction significantly explained variation, and then to determine the significance of the main terms by successively removing each one and comparing the simpler models thus generated to the complex, full model. ANOVA (to check if the means of two or more groups are significantly different from each other) and Tukey’s tests (to assess the significance of differences between pairs of group means) were used to test individual comparisons between treatments.

Dynamic Bayesian Analyses

To investigate the timescale of the effects of Exmoor pony grazing required to manifest under the treatments, a Bayesian model was constructed which quantified (1) lowest threshold of biodiversity in the vegetation community, (2) upper threshold and the (3) rate of transformation of the vegetative community between these two levels given Exmoor pony introduction and removal.

During modelling each treatment covariate, (Grazed, Introduction, Exclusion and Livestock) was characterised in terms of four levels (Exmoors_Now, Exmoors_Past, Livestock_Now and Livestock_Past). Each level took values from 0 to 5 in units of years according to the duration, in years, that each treatment had been applied. Five was chosen as a maximum value, two years longer than the duration of the sampling period. A value of 5 indicates the treatment had been applied for >5 years. It was assumed that any changes effected by the treatment will have reached an equilibrium after 5 years, an assumption that would be violated if an equilibrium took longer to be achieved but was used in the initial modelling as it represented a treatment period greater than that measured during the study. The key advantage of this characterisation of treatments and the associated scoring system is that it made the model sensitive to changes occurring gradually, during the development of the study at different points in time. Details of the model construction are shown in figure 2 while the code is in Appendix 3.

The model was then run twice, using the data collected during the study, initially with the complete data set including all plant species, then using an exclusively flowering plant data subset, to investigate potentially differential results in the magnitude and rates of the effect of the treatment.



Shannon Value has to be between low plateau (Lp) and high plateau (Hp); H controls transition between plateaus

$$Shannon = \overbrace{Lp + (Hp - Lp) * \mathrm{H}}$$

Variable	Meaning
H	Function, change

This keeps H between 0 and 1 (can't have a negative rate)

$$H = \overbrace{\frac{e^L}{1 + e^L}}$$

Variable	Meaning
L	Function, Distance travelled between plateaus

Basically, a linear regression

$$\overbrace{L = a_0 + b * te}$$

0 if Exmoors absent

$$b = (a1 + \overbrace{adE * a2)}$$

$$te = \underbrace{(1 - adE)}_{\text{0 if Exmoors present}} * T - \underbrace{(-1)^{adE}}_{\text{1 if Exmoors absent, -1 if Exmoors present}} * tE$$

Variable	Meaning
adE	Data; Exmoor presence (Y = 1/N = 0)
tE	Data; Time since treatment was applied (0-4 or 4-0)
T	Parameter; Maximum time for equilibrium (2*(-a0)/a1)
a0	Parameter; Baseline (assumes that process starts from 0.01 complete)
a1	Parameter; Rate after removal
a2	Parameter; addition increment/decrement

'on /off switches' are in red

Figure 1.2: Construction of the Bayesian predictive model. Shannon values must fall between the high and low plateau values, which must always be positive. The 'on/off switches' allow the changes in values as Exmoors are added or removed from the grazing treatment area.

2.9 Results

Explorative Principal Component Analysis:

Six functional types of vegetation were observed during the study: graminoids (grasses, sedges, rushes), herbs (flowering plants), heather (woody dwarf shrub), bryophytes (mosses), lichens and tree seedlings. The results of the explorative PCA investigating the differences in treatments driven by abundance of functional types of vegetation, to identify differences under different treatment applications and differences within the treatments across years are shown in figure 3. In figure 1.3:1A and 1B changes over time for each quadrat are shown. PC1 and PC2 additively show that variation in functional types explain 75.7% of the difference between quadrats, with graminoids being the principal drivers of variation (figure 1.3:1C). Changes in proportion of functional type were observed over the duration of the sampling.

At Durnamuck, (figure 1.3:1A) changes were observed under both treatments during the two years of sampling with most of the inclusion treatment samples showing similar directional change. The exclusion treatment was an area left un-manipulated; no grazers were introduced. On Scoraig (figure 1.3:1B) similar, though smaller, directional changes were seen under the inclusion treatment, with little change under the livestock (cattle/sheep) and exclusion treatments. There was more variation amongst the long-term pony grazed plots, although some clustered, others showed temporal directional variation.

Observation of changes under different treatments prompted further analytical investigation.

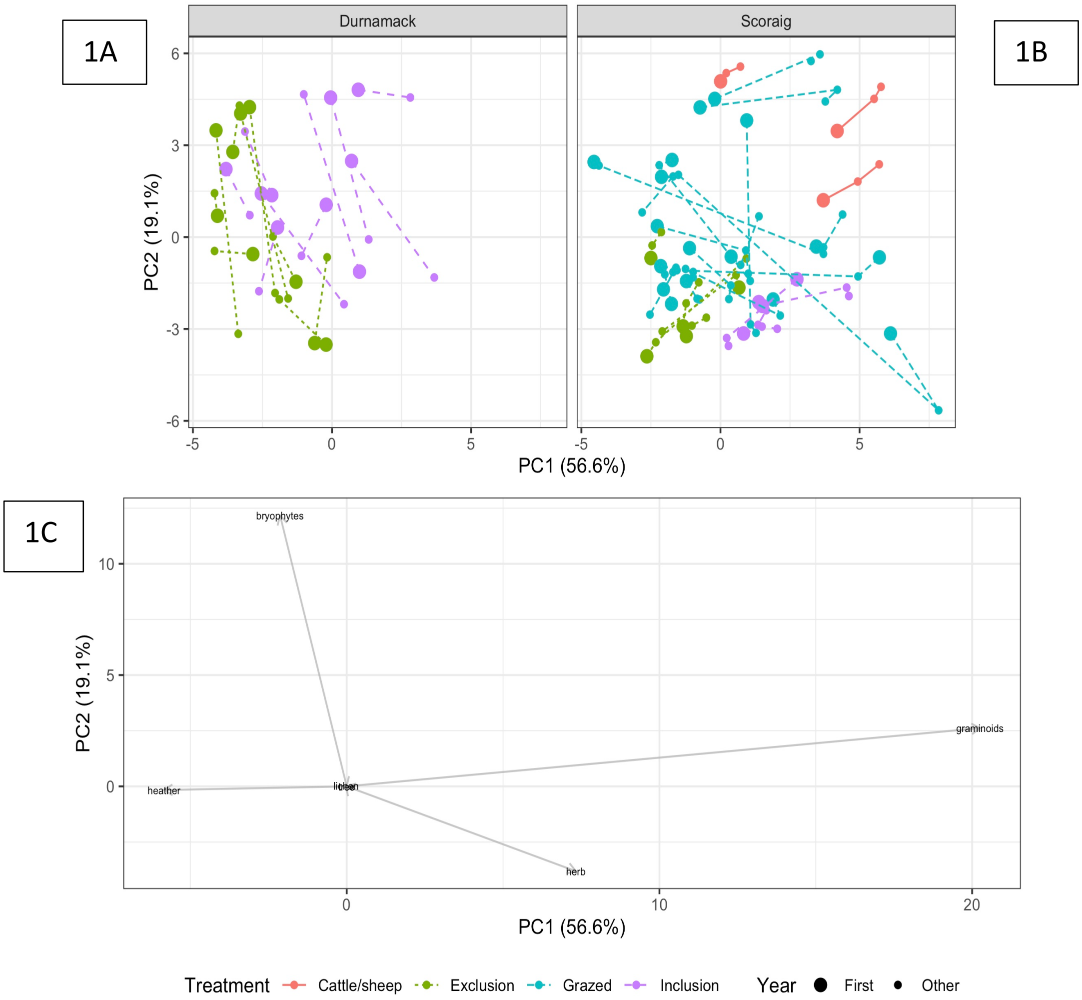


Figure 1.3: Explorative Principal Component Analysis (PCA) by functional types. PCAs show the temporal variation in treatments by site and by functional type.

Shannon's Index:

Two glms had been performed, one with treatment and year and the interaction between them as variables, and one with treatment and year without the interaction, both with Shannon values as the response variables. A likelihood ratio test to determine whether the interaction significantly explained variation between the glms showed the interaction not to be significant. The difference between the degrees of freedom (df) between the two models under comparison was 8df (df = 17 in the full model and 9 in the reduced model). It can therefore be concluded that the long-term pony grazed treatment has a highly significant positive effect (p<0.00001 df = 8). The drivers of this effect are shown by the coefficients from the summary output. The significant results from the output of the reduced model are shown in Table1-2.

Table 1.2: Significant results from GLM with Shannon biodiversity index as response and treatment and year as variables.

glm(formula = SHANNON ~ treat + year, family = gaussian, data = VEG)

Treatment	Coefficient	Standard error	t value	Pr (>|t|)
Grazed	0.194	0.011	17.981	<2e-16***
Exclusion	-0.054	0.017	-3.230	0.00161**
Livestock	-0.063	0.021	-3.053	0.00282**
Year 2022	-0.029	0.014	-2.014	0.04636*

***p<0.001, **p<0.01, *p<0.05

Exclusion on Scoraig showed significantly lower diversity than the unmanipulated long-term pony grazed areas (coefficient = -0.054; standard error = 0.017; t-value = -3.230; p-value = 0.00161). Similarly, the livestock grazed area on Scoraig showed significantly lower diversity than the unmanipulated pony grazed areas (coefficient = -0.063; standard error = 0.021; t-value = -3.053; p-value = 0.00282). Diversity was shown to be slightly lower in 2022 than in other years (coefficient = -0.029; standard error = 0.014; t-value = -2.014; p- value = 0.04636). A least squares ANOVA, although it supported the finding that the treatment was significant, did not find year to have had a significant effect (Table 1.3).

Table 1.3: Results of a least squares ANOVA, response Shannon value, variables treatment and year.

	Df	**Sum of squares**	**Mean squares**	**Mean f-value**	**Pr(>F)**
Grazed treatment	5	0.097	0.020	6.080	4.916e-05***
Year 2022	2	0.015	0.008	2.330	0.102

***p<0.001, **p<0.01, *p<0.05

A post-hoc test Tukey's test, with 95% confidence levels was plotted to visualize the differences in the mean treatment levels (Figure 1.4). It indicated that the cattle/sheep grazed area on Scoraig showed significantly lower diversity than the unmanipulated pony grazed areas. The inclusion treatment on Scoraig showed significantly more diversity than the exclusion treatment. The un-grazed treatment at Durnamuck showed significantly more diversity than the exclusion treatment and the cattle and sheep grazed area on Scoraig.

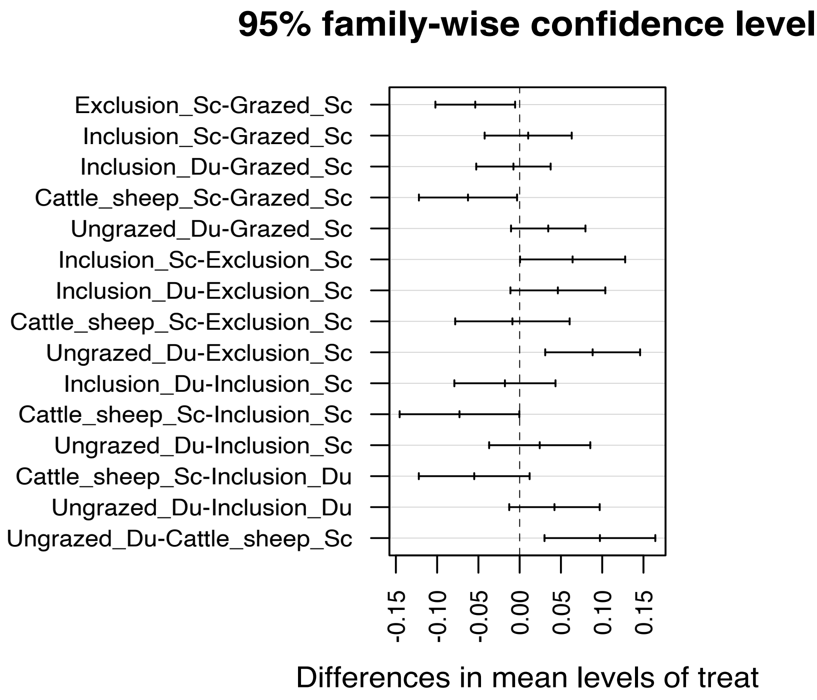


Figure 1.4: Tukey's test results investigating the effect of the treatment on Shannon indices. X axis shows the variation in mean effect of treatment from the 95% confidence level. Y axis shows the treatments under comparison.

Trends in the relative abundance of different plant groups:

Least squares ANOVA tests performed when the individual plant species were allocated into five groups: grasses, sedges, rushes, flowering plants, and mosses all showed very significant responses to the long-term pony grazed treatment, but that year was not significant (Table 1.4).

Table 1.4: Results of ANOVA tests showing the significance of the treatment but not year.

	Df	Sum of squares	Mean squares	Mean f-value	Pr(>F)
Sedges- Grazed treatment	5	61779	12355.8	12.26	1.559e-09***
year	2	3687	1843.4	1.83	0.1652
Grasses- Grazed treatment	5	25087	5017.4	6.25	3.618e-05***
year	2	813	406.7	0.51	0.60
Flowering plants- Grazed treatment	5	25989	3712.7	5.69	1.207e-05***
year	2	1532	765.9	1.17	0.31
Rushes- Grazed treatment	5	25744.5	5148.9	30.31	<2e-16***
year	2	38.3	19.2	0.11	0.89
Mosses- Grazed treatment	5	51221	10244.2	12.40	1.258e-09***
year	2	1958	979.2	1.19	0.31

***p<0.001, **p<0.01, *p<0.05

Sedges

Sedges showed significantly higher abundance under the pony grazed and livestock treatments. The un-grazed treatment had lower abundance of sedges than the livestock or grazed treatments (Table 1.5). The variation in the other comparative means of the treatments was not significant (Figure 1.6).

Table 1.5: Results of Tukey's test showing which treatments had a significant effect on sedges

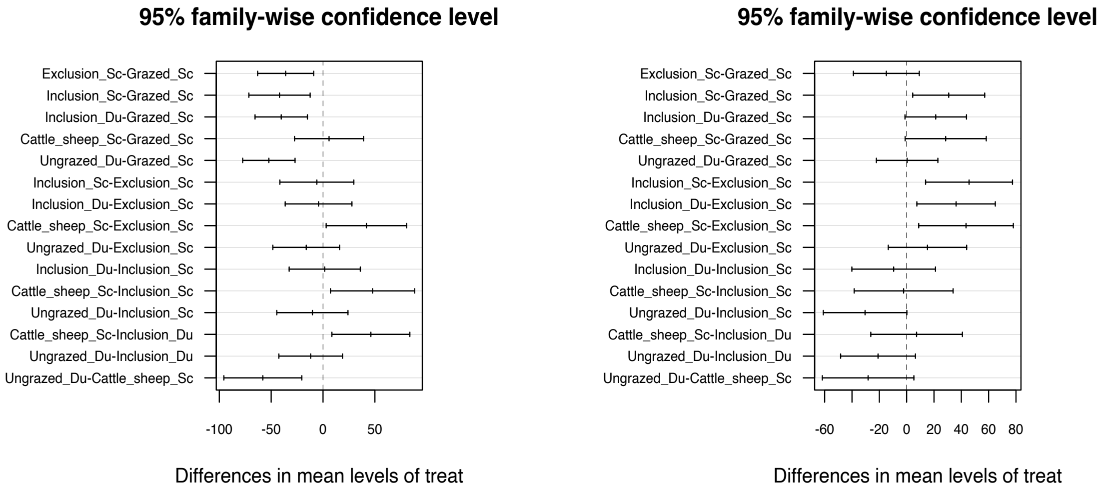
Compared treatments	**Direction of trend**	**Difference in 95% confidence levels**	**Adjusted p-value**
Exclusion/ Grazed	-	-36.00	0.003
Inclusion_Sc/ Grazed	-	-41.95	0.001
Inclusion_Sc/ Inclusion_Du	-	-40.31	<0.001
Un-grazed/ Grazed	-	-52.20	<0.001
Un-grazed/ Cattle_sheep	-	-58.00	<0.001
Cattle_sheep/ Exclusion	+	41.80	0.027
Cattle_sheep/ Inclusion_Sc	+	47.75	0.011
Cattle_sheep/ Inclusion_Du	+	46.11	0.007

Grasses

Compared with the grazed treatment, grasses were significantly more abundant in the Scoraig inclusion area (Table 1.6). Additionally, figure 1.6 shows that the Durnamuck inclusion and the livestock treatments also had almost significantly more abundant grass than the pony grazed treatment. Both inclusion treatments and the livestock treatment had significantly higher grass abundance than the exclusion treatment (Table 1.6). The un-grazed treatment showed significantly lower grass abundance than the Scoraig inclusion (Table 1.6) and almost significantly lower in the Durnamuck inclusion (Figure 1.5).

Table 1.6: Results of Tukey's test showing which treatments had a significant effect on grasses.

Compared treatments	**Direction of trend**	**Difference in 95% confidence levels**	**Adjusted p-value**
Un-grazed/ Inclusion_Sc	-	-30.44	0.052
Inclusion_Sc/ Grazed	+	30.78	0.012
Inclusion_Sc/ Exclusion	+	45.67	0.001
Inclusion_Du/ Exclusion	+	36.17	0.005
Cattle_sheep/ Exclusion	+	43.44	0.005



1A: Sedges

1B: Grasses

Figure 1.5: Tukey's test results investigating the effect of the treatment on 1A: sedges, and 1B grasses. X axis shows the variation in mean effect of treatment from the 95% confidence level. Y axis shows the treatments under comparison.

Flowering plants

The exclusion and livestock grazed treatments showed significantly lower abundance of flowering plants than the pony grazed treatment (Table 1.7). The Durnamuck inclusion treatment had higher abundance of flowering plants than either the livestock or exclusion treatments. The un-grazed treatment had almost significantly higher flowering plant abundance than the livestock treatment (Figure 1.6).

Table 1.7: Results of Tukey's test showing which treatments had a significant effect on flowering plants.

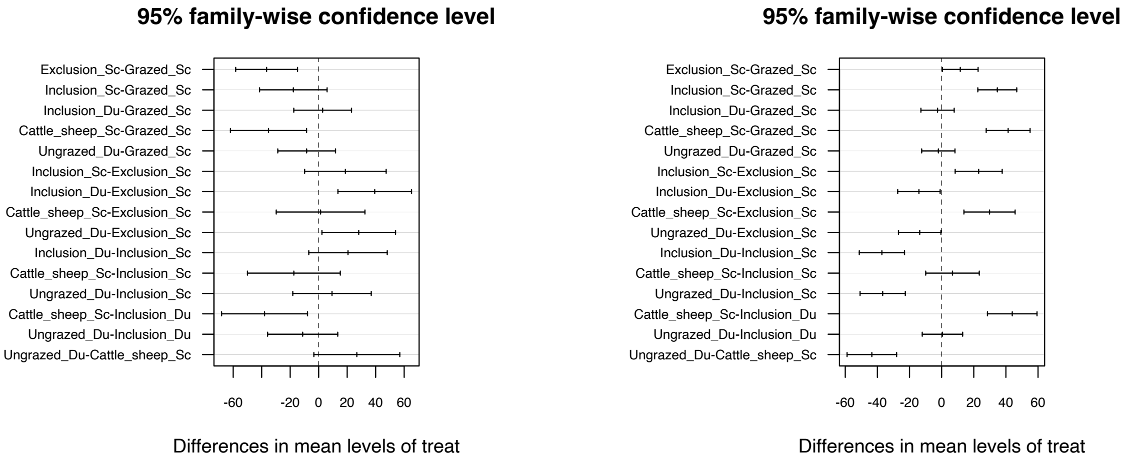
Compared treatments	Direction of trend	Difference in 95% confidence levels	Adjusted p-value
Exclusion/ Grazed	-	-36.55	0.000
Cattle_sheep / Grazed	-	-35.22	0.002
Cattle_sheep/ Inclusion_Du	-	-38.00	0.005
Inclusion_Du/ Exclusion	+	39.33	0.000
Un-grazed/ Exclusion	+	28.11	0.024

Rushes

The exclusion, inclusion and cattle grazed treatments showed significantly higher abundance of rushes than the pony grazed treatment (Table 1.8). The inclusion treatment showed significantly greater abundance of rushes than the exclusion treatment (Table 1.8). The livestock grazed treatment showed significantly greater rush abundance than either the exclusion or the Durnamuck inclusion treatments (Table 1.8). The un-grazed treatment showed significantly lower rush abundance than the exclusion, inclusion or cattle grazed treatments (Table 1.8). The Durnamuck inclusion treatment showed significantly lower rush abundance than the exclusion or exclusion treatments on Scoraig (Table 1.8). Other pairwise treatment comparisons were not significant (Figure 1.8).

Table 1.8: Results of Tukey's test showing which treatments had a significant effect on rushes.

Compared treatments	Direction of trend	Difference in 95% confidence levels	Adjusted p-value
Inclusion_Du/ / Exclusion	-	-14.14	0.028
Un-grazed/ Exclusion	-	-13.64	0.039
Inclusion_Du/ Inclusion_Sc	-	-37.19	0.000
Un-grazed/ Inclusion_Sc	-	-36.69	0.000
Un-grazed/ Cattle_sheep	-	-43.44	0.000
Exclusion/ Grazed	+	11.59	0.035
Inclusion_Sc / Grazed	+	34.64	0.000
Cattle_sheep/ Grazed	+	41.39	0.000
Inclusion_Sc / Exclusion	+	23.05	0.000
Cattle_sheep/ Exclusion	+	29.80	0.000
Cattle_sheep/ Inclusion_Du	+	43.94	0.000



1A: Flowering plants

1B: Rushes

Figure 1.6: Tukey's test results investigating the effect of the treatment on 1A: flowering plants, and 1B rushes. X axis shows the variation in mean effect of treatment from the 95% confidence level. Y axis shows the treatments under comparison.

Mosses

The Scoraig inclusion treatment showed significantly lower moss abundance than the pony grazed treatment (Table 1.9). The Durnamuck inclusion and the livestock treatments showed significantly greater abundance of moss than the pony grazed treatment (Table 1.9). The Durnamuck inclusion, livestock, and un-grazed treatments showed significantly greater abundance of moss than the Scoraig inclusion treatment (Table 1.9). The livestock and un-grazed treatments showed significantly greater abundance of moss than the exclusion treatment (Table 1.9). The exclusion treatment nearly showed significantly lower moss abundance than the grazed treatment (Figure 1.7). The un-grazed treatment nearly showed significantly lower moss abundance than the livestock and the Durnamuck inclusion treatments (Figure 1.7).

Table 1.9: Results of Tukey's test showing which treatments had a significant effect on mosses.

Compared treatments	Direction of trend	Difference in 95% confidence levels	Adjusted p-value
Inclusion_Sc / Grazed	-	-36.00	0.003
Inclusion_Du/ Grazed	+	32.90	0.001
Cattle_sheep/ Grazed	+	33.90	0.018
Inclusion_Du / Exclusion	+	52.60	0.000
Cattle_sheep/ Exclusion	+	53.60	0.000
Un-grazed/ Exclusion	+	29.88	0.041
Inclusion_Du/ Inclusion_Sc	+	68.00	0.000
Cattle_sheep/ Inclusion_Sc	+	69.00	0.000
Un-grazed/ Inclusion_Sc	+	45.28	0.001

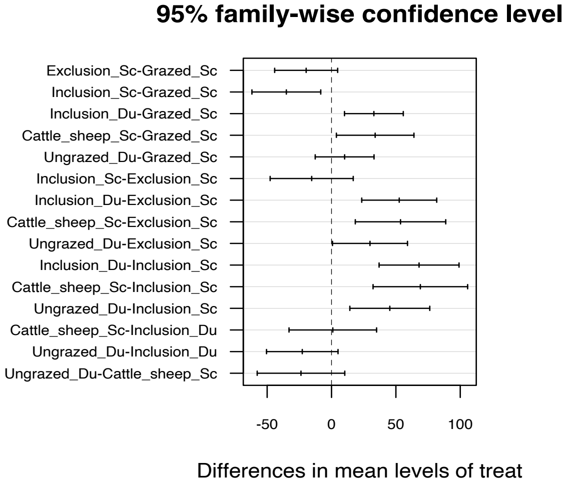


Figure 1.7: Tukey's test results investigating the effect of the treatment on mosses. X axis shows the variation in mean effect of treatment from the 95% confidence level. Y axis shows the treatments under comparison.

The changes in the Shannon biodiversity and in the relative abundancies of the grouped species by year are shown in figure 1.8. The Shannon indices declined under all treatments on Scoraig between 2020 and 2022 Figure 1.8). The Durnamuck inclusion showed a similar declining trend between 2020-2021, whilst the un-grazed treatment at Durnamuck showed a positive change in Shannon value (Figure 1.8). Sedges showed little change in abundance throughout the study except for increased abundance under the pony grazed and livestock treatments in 2022 (Figure 1.8). Grasses showed slightly increased abundance throughout the study across all treatments, especially under the Scoraig inclusion treatment in 2022 (Figure 1.8). Rush abundance rose during the study period under the exclusion, livestock, and un-grazed treatments, declined slightly under the pony grazed treatment, but showed the greatest decline under the Scoraig inclusion treatment, where they had shown the highest abundance at the commencement of the study (Figure 1.8). The Durnamuck inclusion showed no change in rush abundance (Figure 1.8). Flowering plant abundance showed little variation throughout the study except for an increase under the pony grazing treatment in 2022 and a slight increase under the Scoraig inclusion treatment, also in 2022 (Figure 1.8). Moss abundance also showed little variation except for increasing during 2020-2022 under the exclusion treatment and increasing slightly in 2022 under the livestock and inclusion treatments.

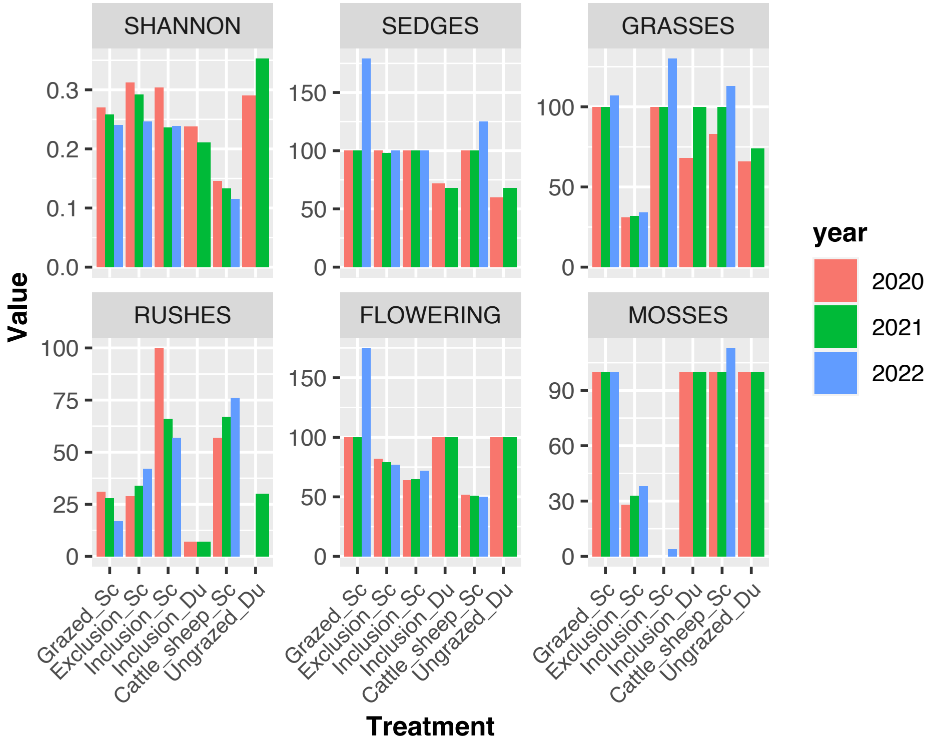


Figure 1.8: The changes in overall biodiversity (Shannon) and in species group abundance by year (colour). Treatments are shown on the x-axis, abundance on the y-axis.

Dynamic Bayesian Analyses:

Investigation into the timescale of the effects of Exmoor pony grazing required to manifest under the treatments using a Bayesian model which quantified (1) lowest threshold of biodiversity in the vegetation community, (2) upper threshold and the (3) rate of transformation of the vegetative community between these levels, upon Exmoor pony introduction and removal was constructed.

Figure 1.9 shows the simulated effects of the introduction of Exmoor ponies on vegetation and demonstrated what would be expected during different phases of impacts: 1) a rise in the rate of increase from the initial specified level of biodiversity present before the introduction of ponies (Lp) over the five-year period specified in the model, towards the maximum specified level of biodiversity expected over the five year period following introduction of ponies to the system , the high plateau (Hp); and 2) the predicted rate of decrease back to the low plateau as an effect of removal of Exmoor ponies. Figure 1.9 shows an initial peak in biodiversity, the high plateau, one year after their removal, followed by a decline towards the low plateau in the subsequent four years.

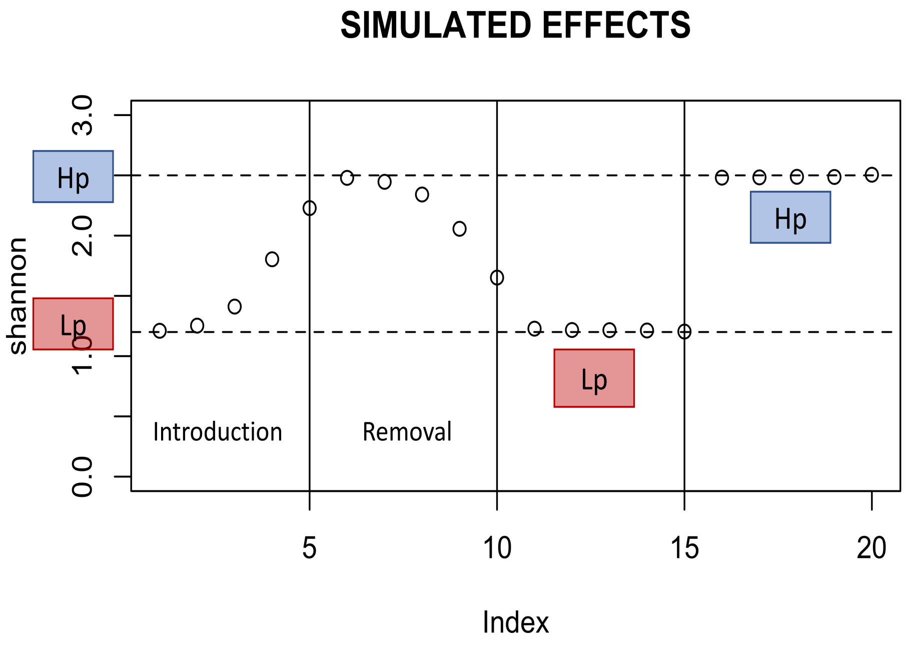


Figure 1.9: Bayesian simulation model of the effect of addition and removal of Exmoor ponies on Shannon diversity values. The x axis index represents the time since, in years, since the introduction (0-5) or removal (5-10) of Exmoor ponies (0-5), the removal of Exmoor ponies (5-10), then for comparison, the biodiversity Lp (10-15) and the biodiversity Hp (15-20). The y axis, Shannon biodiversity index values, shows Lp, the low plateau of predicted values (years 10-15) and Hp, the high plateau of predicted values and (years 15-20). Lp, the low plateau of predicted values.

The model was then run twice, using the data collected during the study, initially with the complete data set including all plant species, then using an exclusively flowering plant data subset, to investigate potentially differential results in the magnitude and rates of the effect of the treatment. The models (Figure 1.10) showed that the rate of change in Shannon's diversity from the Lp to the Hp was four years for both the total species data (rate of increase 0.025) and for the specifically flowering plant subset (rate of increase 0.09). However, no reduction in the Shannon values was predicted for either the complete data or the flowering plant subset during a five-year period following the removal of Exmoor ponies.

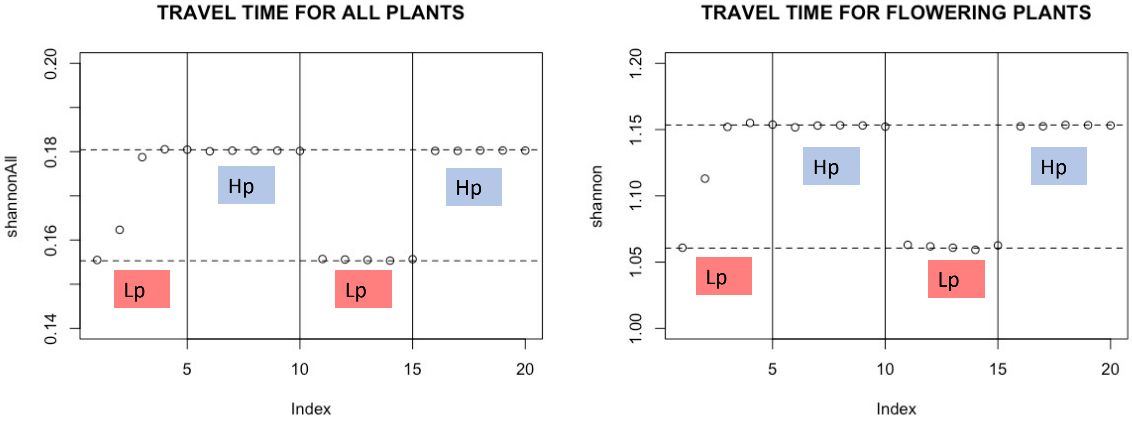


Figure 1.10: Bayesian models of the effect of addition and removal of Exmoor ponies on Shannon diversity values on a complete data set including all the plants present and a subset including only flowering plants. The x axis index represents the time since, in years, since the introduction of Exmoor ponies (0-5), the removal of Exmoor ponies (5-10), then for comparison, the biodiversity Lp (10-15) and the biodiversity Hp (15-20). The y axis, Shannon's biodiversity index values, show Hp, the high plateau of predicted values and Lp, the low plateau of predicted values.

2.10 Discussion

This study shows that the long-term pony grazed treatment has a highly significant positive effect on the Shannon biodiversity ($p<0.00001$ df = 8) when compared to an area long-term grazed by commercial livestock. Furthermore, exclusion of Exmoor ponies from an area previously grazed by them showed a significant reduction in the Shannon biodiversity index showed significantly lower diversity than the unmanipulated pony grazed areas (coefficient = -0.054; standard error = 0.017; t-value = -3.230; p-value = 0.00161), further re-enforcing the finding that their grazing has a positive impact on overall biodiversity. In a study of 162 plots in Finland, Pykälä et al. (2005) report a decline in species richness in plots un-grazed for>10 years when compared to grazed plots, indicating that overall grazing rather than non- grazing is beneficial to grassland biodiversity, although this study did not investigate differences in impact between different species of grazers. The findings of the study by Pykälä et al. (2005) are re-enforced by Henning et al. (2017) who report an increase in species richness on plots grazed by mixed horses and cattle over heathland plots previously un-grazed for 7 years. Bonavent et al. (2023) report that during a five-year experiment, with five grazing treatments applied to sets of 5 x 9 m plots, both year-round and seasonal grazing by a mixture of Galloway cattle and Exmoor ponies increased the alpha- diversity of semi-natural grassland plots, whereas exclusion plots showed an increase in vegetative litter and the lowest species richness of all the grazing treatments, although differences between the grazing effects of the Galloways and Exmoors were not explored in this study. Both Galloways and Exmoors are however used as ancient-species analogues so the impact of their joint grazing might be different to that of grazing commercial livestock. Overall, it appears that grazing has a more positive effect on grassland biodiversity than exclusion of grazers, and further that grazing by Exmoor ponies has an especially positive impact.

The explorative PCA using functional groups of vegetation indicated that graminoids were the principal drivers of the differences in abundance of functional types of vegetation. ANOVA tests performed investigating the effects of the grazing treatments on the functional groups: grasses, sedges, rushes, flowering plants, and mosses, all showed very significant responses to the treatments. Grasses as drivers of the differences between treatments may be supported by the ANOVA results. Grasses were significantly more abundant in the Scoraig inclusion than in the long-term pony grazed treatment areas. The Durnamuck inclusion and the livestock treatments had almost significantly more abundant grass than the pony grazed treatment. Both the Scoraig and Durnamuck inclusion treatments and the livestock treatment had significantly higher grass abundance than the exclusion treatment where ponies had been recently excluded. These results indicate that ponies are likely to preferentially graze grasses rather than other functional groups, lowering their relative abundance. That the Durnamuck inclusion and the livestock grazed treatments had higher graminoid abundance than the exclusion treatment indicates that livestock do not exert as high selection pressure on grasses as ponies, and that the recent introduction of ponies had not yet reduced graminoid abundance to the level on areas where they grazed long-term. However, the Durnamuck un-grazed treatment showed significantly lower grass abundance than the Scoraig inclusion and almost significantly lower than the Durnamuck inclusion suggesting that long-term absence of all grazing animals does not lead to graminoid dominance. These findings indicate that grazing will influence functional group abundance and is likely to promote diversity of functional groups. Dietary analysis of ponies on Exmoor (Baker 2008) and of cattle and ponies in the New Forest, England (Putman et al. 1987) show there is strong preferential grazing of grasses by ponies, particularly in summer, supporting the finding that graminoids are the drivers of the variation in functional group abundance.

ANOVA tests showed that the long-term pony grazed treatment had a highly significant positive effect on flowering plant abundance (*f*=5.69, p= 1.207e-05***). The exclusion and livestock grazed treatments showed significantly lower abundance of flowering plants than the long-term pony grazed treatment, indicating that grazing by commercial livestock does not promote flowering plant abundance when compared to grazing with Exmoor ponies and further, that the removal of the ponies reduces forbe abundance. The Durnamuck inclusion treatment, where ponies were recently introduced, had higher abundance of flowering plants than either the livestock or exclusion treatments, indicating that the positive effect of pony introduction on forbe abundance was rapidly apparent. That the un-grazed treatment had almost significantly higher flowering plant abundance than the livestock treatment indicated that no grazing is likely to be more beneficial to flowering plant abundance than grazing by commercial livestock, although not as beneficial as grazing by Exmoor ponies. However, at Oostvaardersplassen, Netherlands, a 3-year grazer exclusion experiment led to a decline in flowering plant species on long-term grazed pastureland (van Klink, Ruifrok, and Smit 2016). This might suggest that the 3-year period did not allow the flowering plant diversity to re-equilibrate after the removal of grazers, in contrast to this study where the un-grazed area had been allowed >15 years to adapt to non-grazing.

The finding of increased flowering plant abundance in areas grazed by Exmoor ponies but not commercial cattle and sheep is concordant with other published studies. Numerous studies report that cattle consume more forbs than equines ((Fraser, Stanley, and Hegarty 2019); (Putman et al. 1987); (Fleurance et al. 2012); (Menard et al. 2002)). A meta-analysis by Tälle et al. (2016) found a reduction in diversity of forbes in some areas grazed by sheep. Fleurance et al. (2012) and Menard et al. (2002) both found that as equines are essentially grass feeders, cattle generally consume forbs and legumes. Fleurance et al. (2012) describe examples of equine grazing improving species richness, especially of flowering plants, by reducing inter-species competition by preferential grazing of prolific graminoids from Dutch sand dunes. Fraser, Stanley, and Hegarty (2019) and Putman et al. (1987) both report reduced competition from the invasive grass, *Molinia caerulea,* on sites grazed by ponies. It comprised 20% of their diet in summer, and there was an observed increased forb abundance due to reduced graminoid competition. Whether the equines in these most of the studies are ancient analogue or modern breeds is not reported, although in the Putman et al. (1987) study they are New Forest ponies, a breed native to the study area in southern England. In contrast, Critchley et al. (2008) reports that during a 4-year study investigating heathland grazing restoration in the UK, sheep grazing did not reduce *Molinia caerulea* abundance and no significant restoration effects were recorded. Fleurance, Dumont, and Farruggia (2010) report that a 4-year study of continuous grazing by equines on the plant diversity in Limousine, France, resulted in the creation of relatively stable lawns of short grasses, which enabled flowering plants to compete with taller vegetation. They report that flowering plant abundance increased from an average of 4% to 16% of the plot area. That these lower canopy height lawns created by grazing equines show higher flowering plant abundance was also reported from grasslands in Sweden (Garrido et al. 2019) and heathlands in Germany (Henning et al. 2017). This effect of the removal of taller plant species, particularly rushes, creating short vegetation lawns benefitting forbes was observed in this study (Appendix 4). Bonavent et al. (2023) reported increased proportions of forbes to graminoids in plots grazed by Exmoor ponies and Galloway cattle, with the reverse trend in the mown plots. This suggests that selective removal of vegetation rather than complete removal of biomass by mowing benefits flowering plant abundance, a very relevant finding for grassland managers, as the cost effectiveness of mowing *vs* grazing may be a consideration on restoration sites.

These reports, that livestock rather than Exmoor ponies are more likely to consume flowering plants, less likely to remove competitive invasive plants and less likely to create short grass lawns that favour flowering plant abundance support this study's finding of reduced biodiversity and flowering plant abundance in the under the livestock grazing treatment. Interestingly, Cutter et al. (2022) report that flowering species richness and abundance were significantly higher in cattle pastures than sheep pastures. Since Bonavent et al. (2023) report increased diversity in plots grazed by Galloways and Exmoor ponies and in this study there was reduced diversity under the livestock treatment, grazed by both commercial sheep and cattle vs Exmoor ponies, further studies could investigate possible differences in the effects of grazing by (1) Exmoor ponies; (2) a modern pony breed; (3) commercial cattle; (4) ancient analogue cattle; (5) commercial sheep and (6) ancient analogue sheep to elucidate potential differences in effects between both the species of grazer and the commercial *vs* ancient breeds.

These results of this study, that there is improved biodiversity and flowering plant abundance in areas grazed by Exmoor ponies are supported by Vodičková et al. (2019) and Konvicka et al. (2021) where the addition of grazing Exmoor ponies to sites was observed to improve the floristic abundance and thus improved the habitat for butterflies, the target of the conservation effort. The use of grazing animals to improve habitat diversity with consequent positive 'knock on' effects for vegetative and invertebrate abundance is increasingly widespread. A 5-year study by Köhler, Hiller, and Tischew (2016) that found that grazing by Konik horses, also used as an ancient-analogue breed, was beneficial to orchid abundance by creating short grass lawns, a finding that results from this study would support (Appendix 4).

The results of predictive Bayesian modelling of the effects of Exmoor pony grazing suggest similar results to those found in the statistical testing. When a data set of all the plants present during the study and a subset of the flowering plants were modelled using a Bayesian predictive model, there was a suggested increase in Shannon values of 0.025 for all the species present and of 0.09 for flowering plant subset. The effects of the inclusion of Exmoor ponies manifested rapidly, reaching an equilibrium after about 4-5 years. However, the model suggested that effect persisted after Exmoor ponies are removed. This contrasts with the findings from the ANOVA tests that overall biodiversity and flowering plant abundance were both significantly lower under the exclusion treatment than under the long-term pony grazed treatment. Further investigation to test whether the effect indicated by the Bayesian model could be supported by experimental evidence in other studies, would, if it was supported, have interesting implications for grassland management. If even the relatively short-term (4-5 years) use of Exmoor ponies on sites to improve biodiversity and flowering plant abundance could have persistent effects on sites it might encourage their use on sites where the long-term use of grazing animals in site management might not be possible. An investigation into the cause of the persistent effect, if present, would usefully inform the management of grassland sites.

These results suggest that Exmoor ponies may have an important role to play in nature-based solutions to the biodiversity and climate change crises. Grasslands and open canopy woodlands, grazed by appropriate herbivores to maintain high vegetative alpha diversity, overall species richness, and the functioning ecosystem services associated with this diversity, will have the potential to play an important part in the restoration of biodiversity, carbon storage and thus contribute to climate mitigation strategies ((Bonavent et al. 2023); (Schmitz et al. 2023);(Tree 2023); (Tree 2019); (Malhi et al. 2022); (Chang et al. 2021)). Being hind-gut fermenters, methane is less problematic with ponies than with ruminant livestock (Cromsigt et al. 2018). This study found that that their grazing habit encouraged an increase in forbes over grasses, a result that Chang et al. (2021) suggests corresponds with higher soil carbon storage. Indeed, 297 Exmoor ponies managed by The Moorland Mousie Trust are already used in conservation grazing in the UK in 2023, on 55 sites, with total acreage more than 8735.5 acres. These sites are managed on behalf of Wildlife Trusts, County Councils, the National Trust, and private owners, including sites of public interest including The White Cliffs of Dover, Kent, and Land's End, Cornwall (personal email communications from Juliet Rodgers, Moorland Mousie Trust, 28/4/23, and Sue Baker, Exmoor Pony Society, 16/7/23). A further 51 sites, including Knepp Rewilding in Sussex also graze Exmoor ponies due to their value as eco-engineers (Tree (2019) and personal email communication from Sue Baker, Exmoor Pony Society, 16/7/23).

Utilisation of Exmoor ponies in rewilding and restoration schemes is also prevalent across Europe, where they are used as ancient equine analogues where trophic rewilding is practiced including in the Netherlands, Denmark, and the Czech Republic. In 2023, 602 Exmoors are used in this role by 16 organisations in the Netherlands (personal email communication from Hans Hovens, Chairman Samenwerkingsverband Exmoorpony, 16/5/23). At Mols Reserve in Denmark 26 Exmoor ponies graze the rewilding site studied by Bonavent et al. (2023). Other herds of Exmoor ponies in the Czech Republic were the subjects of other studies cited above ((Konvicka et al. 2021); (Vodičková et al. 2019)).

The positive effect on biodiversity and on flowering plant abundance associated with the grazing of Exmoor ponies, as found in this study, may be in part due to their retention of primitive trait combinations lost in modern breeds ((Baker 2008); (Hovens 2014); (Tree 2019); (Hagstrup et al. 2020)). Lundgren et al. (2020) state that megaherbivores retaining these ancient characteristics are more likely engineer restoration of ecosystem functions. The diverse landscapes that they create may contribute to mitigation of current anthropogenic climate change (Cromsigt et al. 2018). Therefore, these results would suggest that if the aim of a conservation grazing project is to increase biodiversity and especially the abundance of forbes, often a target of ecological restoration projects, the introduction of Exmoor ponies and the removal of other commercial livestock will increase the likelihood of achieving that goal.

2.11 Acknowledgements

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Chapter 3: The impact of parasite load and seasonality on body condition in a herd of free-living Exmoor ponies

3.1 Abstract

Free-living Exmoor ponies are widely used as ecological engineers on conservation grazing sites and in rewilding projects throughout the UK and Europe. In these situations, they are not usually given anthelmintic treatment, or supplementary feeding in winter, and so are subject to the challenges of parasite loads and of seasonal variation in vegetation quality and abundance, and the increased energy demands of winter weather. However, few studies have directly assessed whether this relaxed husbandry could have impacts on body condition, which could compromise the potential for low maintenance rewilding. This study aimed to investigate the impact of seasonality and parasite load on the body condition scores of free-living Exmoor ponies~~.~~ Body condition scoring and faecal egg counts were performed in spring and autumn. Resource limitation over winter correlated with significantly lower spring than autumn CS (spring CS= -1.22, p=<2e-16). Ponies consistently gained >1 CS point in summer compared to spring scores. The FEG levels, whilst significantly affecting CS were generally lower than reported in other studies of feral horses consistently averaging CS = -0.218/ 100FEG (r=2.178e-03, p=3.15e-05). Parasites were over-dispersed, infection varying from 18.5% to 37.5%. Gold's (2019) feral horse study reported that FEG load capacity was moderately heritable (h^2 =0.43, +/- 0.11. These relatively low FEG scores compared with other studies suggests that if further investigation showed this trait to be present in Exmoors, breeders could select for it. Results from this study indicate that in a natural seasonal cycle, Exmoors use stored body reserves during periods of resource limitation in winter but can successfully replenish these reserves when resources are not limited in summer.

3.2 Introduction

Gastrointestinal parasites are a global issue affecting both domestic livestock and wildlife (Charlier et al. 2020). Nematode parasites have always been co-existing with plants and animals since early times. The earliest fossil vertebrate-parasitic nematodes are found in fossilised Upper Triassic coprolites (Poinar 2015). Domesticated horse remains from the Roman period have been found to carry loads of gastrointestinal parasites (Jones, Hutchinson, and Nicholson 2015) and have remained an economic burden in modern livestock production. Charlier et al. (2020) investigated the cost of the major helminth infections in domestic ruminants across18 European countries and estimated losses to the livestock industry of € 1.8 billion. Most of these costs, 81%, were associated with lost production (reduced milk yield, fertility, growth rates) while the remaining 19 % was attributed to the cost of treating the infections. Within this estimate, losses associated with anthelminthic resistant gastrointestinal nematode infections was estimated to be € 38 million annually. Gastrointestinal parasites can have a significant impact on the hosts' development, condition, health, reproduction, and longevity (Debeffe et al. 2016).

The routine use of domestic horses in sport and leisure horses is widespread in Europe, the USA and Australasia and the economic burden of gastrointestinal parasites adds substantially to maintenance costs, with veterinary practices offering annual monitoring using faecal egg counts and de-worming programs (for example, at https://www.rossdales.com/hertfordshire/services/worming, accessed at 13.08 on 16/8/23, the suggested costs for 2023 are £66 - £110 per horse). These costs can be prohibitive, particularly in countries where the economic impact of the reduced performance of working equines, critical to the livelihood of their owners, can be considerable (Waqas et al. 2015; Tahir et al. 2016; Tamene, Bayu, and Wondimu 2019; Imani-Baran et al. 2019).

Although gastrointestinal parasites have long been a recognised cause of disease in domestic horses, prior to the introduction of benzimidazole anthelmintics in the 1960s they were not well controlled (Kaplan and Nielsen 2010). The small strongyles of horses, cyathostomins, are the most prevalent and pathogenic parasites of horses worldwide (Corning 2009). Eggs are passed in faeces and the infection of horses is by ingestion of infective third-stage larvae from the environment, larval development occurring within a few days (Corning 2009). Cyathostominosis, the mass emergence of cyathostomins, can have a high fatality rate even with modern veterinary care (Corning 2009). In the 1960s veterinary advice was to treat domestic equines every eight weeks, a strategy that rapidly resulted in reduced morbidity and mortality and with associated improvement in equine health and performance (Kaplan and Nielsen 2010). This practice continued until the turn of the millennium, with rotation of drug classes being practiced as new, different drugs became available. Prevention of cyathostominosis and parasite related colic relied on the repeated use of anthelmintic treatments throughout the life of a horse (Corning 2009; Lester et al. 2013). Unfortunately, this routine dosing, not always accurately calibrated or administered, resulted in increased anthelmintic resistance in parasitic nematodes (Kaplan and Nielsen 2010). Since parasites are usually over-dispersed in their host population applying the same anti-parasite treatment to all horses in a herd, without knowledge of the parasite load of the individuals, the species of parasites present, and without considering the efficacy of the drugs against the specific parasites present, as was widely advised until about a decade ago, exacerbated the resistance problem (Kaplan and Nielsen 2010; Lester et al. 2013).

Regular, routine de-worming had been an effective solution to the problem of parasite loads for several decades, but with increasing resistance issues, solutions other than anthelmintic treatment to control parasitism and its effects on host animals needed to be explored (Williams 1997). To counter the resistance problem horse owners are now offered different advice for herd de-worming management. Faecal worm egg counts (FEG) are now increasingly used as a targeted approach to parasite control (Lester et al. 2013). FEG are performed on all animals in the herd to determine individual parasite loads, so that only those individuals with high numbers of worms are treated with an anthelmintic. The aim of this is to reduce environmental contamination with anthelmintic residues, and to leave a proportion of the worm population unexposed to selection pressure for anthelmintic resistance in some individuals (Lester et al. 2013). However, although problems caused by the widespread, sometimes indiscriminate use of anthelmintics leading to resistant parasites causes a direct problem for livestock breeders are widely acknowledged, there are other, indirect problems associated with parasite control using medication.

One of these indirect problems is the effect of these medications on invertebrate life. The residues of the anthelmintics used to control the gastrointestinal parasites of livestock, are excreted largely un-metabolised in faeces. These residues can have toxic effects on dung-colonising insects (Strong and Wall 1990). For example, Strong and Wall (1990) showed that dung from cattle treated with a slow-release ruminal ivermectin bolus remained almost arthropod-sterile for more than 3 months. Reduction of coprophagous beetles inhibits the process of dung recycling and thus may enhance the persistence of dung-dwelling parasitic stages (Sands and Wall 2017). The disappearance and mineralisation of dung, limiting pasture fouling and cycling nutrients is essential in sustainable grazing systems (Svendsen et al. 2005). Dung beetles and dung flies play a key role in the break-up of surface dung, and earthworms are the main agents responsible for removal and transport of dung into the soil (Svendsen et al. 2005). Ivermectin in dung constitutes a potential risk for dung breeding insects and for soil invertebrates partly living in and around dung (Jensen, Krogh, and Sverdrup 2003), which could compromise the critical ecosystem services that they provide: reduced pest flies; reduced gastrointestinal parasites; reduced pasture fouling and increased cycling of soil nutrients (Beynon, Wainwright, and Christie 2015). The value of dung beetles to the UK has been estimated as £367 million each year (Beynon, Wainwright, and Christie 2015), therefore reduction of anthelmintics could not only reduce resistance of parasites but could also reduce impacts on ecosystem services provided by insects such as dung beetles.

An important aim of rewilding and restoration projects is often to improve the functioning of natural ecosystems, without relying on chemical fertilisers or drugs to combat pests and parasites (Tree 2019; Tree 2023). Large herbivores that can be maintained without regular treatments of worms, for example, would thus be highly beneficial. However, an investigation into whether Exmoor ponies that have not been treated will suffer from negative impacts on body condition, which could compromise their ability to survive harsh environmental conditions would be required.

Parasites are of course, not the only factor that affects the body condition score (CS) of animals. The CS of an animal depends on the balance between energy intake and expenditure (Rudman and Keiper 1991). If energy expenditure exceeds energy intake the animal will lose condition and vice versa. CS can be affected by the quantity and quality of food available, weather conditions, age, life stage and reproductive status (Rudman and Keiper 1991). The CS of free-living equines is therefore likely to be lowest in spring after the period of increased energetic demands associated with severe weather combined with reduced and poorer quality food availability outside the vegetation growing season (Rudman and Keiper 1991). Equines, as opposed to ruminants, being hind gut fermenters, can utilise poor quality vegetation in winter due to the cellulose-fermenting symbionts in their hindgut (Kuntz, 2006). Able to absorb more nutrients from food with a high fibre content than ruminants, equines, including New Forest and Exmoor ponies, cope with seasonal variation in food quality by adjustment of their diets and increased dry matter intake to maintain their CS (Putman et al. 1987; Gates 1979), but are nevertheless likely to show a natural variation (Slivinska et al. 2020). Gill (1987) in a study of 200-400 live and 190 dead New Forest ponies from free-living herds in the New Forest, Southern England, showed that deterioration in CS over winter was availability and severe weather, leading to an excess of energy output over intake. In late spring, improvement in the CS of non-lactating ponies was observed, concurrent with improved weather and vegetation growth, with peak CS scores being attained by all ponies in autumn. However, another study of the same equine population showed similar findings, but additionally found dentition, and parasitic damage to the liver to also influence CS significantly (Putman et al. 1987).

There also could be an interaction between seasonality and parasite loads on CS. For example, studies of three feral horse populations in the USA and Canada, Assateague Island (Rudman and Keiper 1991), Shackleford Banks (Rubenstein and Hohmann 1989) and Sable Island (Debeffe et al. 2016; Jenkins et al. 2020) reported negative covariance between CS and FEG, with winter resource limitation particularly influencing the incidence of poor CS in lactating mares in spring, which experience particularly high energy demands. Variation in parasite loads among individuals means that the impacts of seasonality also might vary within a herd. For example, the feral horse population in Shackleford banks, showed overdispersion of parasites, with 25% of the herd showing no evidence of parasites based on FEGs but others having extremely high loads (FEG = 50,000 – 76,000), which was correlated negatively with age and home range size and positively with herd density (Rubenstein and Hohmann 1989). The Sable Island horse population, unmanaged since 1960, also showed similar patterns of response to seasonality and FEG (Debeffe et al. 2016). Such high parasite loads could Parasites are of course, not the only factor that affects the body condition score (CS) of animals. The CS of an animal depends on the balance between energy intake and expenditure (Rudman and Keiper 1991). If energy expenditure exceeds energy intake the animal will lose condition and vice versa. CS can be affected by the quantity and quality of food available, weather conditions, age, life stage and reproductive status (Rudman and Keiper 1991). The CS of free-living equines is therefore likely to be lowest in spring after the period of increased energetic demands associated with severe weather combined with reduced and poorer quality food availability outside the vegetation growing season (Rudman and Keiper 1991). Equines, as opposed to ruminants, being hind gut fermenters, can utilise poor quality vegetation in winter due to the cellulose-fermenting symbionts in their hindgut (Kuntz, 2006). Able to absorb more nutrients from food with a high fibre content than ruminants, equines cope with seasonal variation in food quality by adjustment of their diets and increased dry matter intake to maintain their CS (Putman et al. 1987; Gates 1979)(Kuntz, 2006), but are nevertheless likely to show a natural variation (Slivinska et al. 2020). Gill (1987) in a study of 200-400 live and 190 dead free-living ponies in the New Forest, Southern England, showed that deterioration in CS over winter was availability and severe weather, leading to an excess of energy output over intake. In late spring, improvement in the CS of non-lactating ponies was observed, concurrent with improved weather and vegetation growth, with peak CS scores being attained by all ponies in autumn. However, another study of the same equine population showed similar findings, but additionally found dentition, and parasitic damage to the liver to also influence CS significantly (Putman et al. 1987).

However, there also could be differences in tolerance to parasite loads that might make some individuals or breeds more robust than others to reduced husbandry practices. In domesticated horse populations de-worming treatment is recommended for individuals with a FEC of 200+/g but the untreated Sable Island populations had over three times this load in both dead (FEG average = 666 eggs per gram) and live (FEG average = 689 eggs per gram) horses (Jenkins et al. 2020), with the highest loads associated with immature and lactating animals (Debeffe et al. 2016), suggesting that the energy demands of growth and lactation compromised the energy budget available for immune response to parasites. Based on visual inspection of the entire animal to assess the amount of fat, quantified in evenly spaced intervals of 0.5 between the lowest and highest scores (Carroll and Huntington 1988), a multi-year study of feral horses in Argentina found that adult mares were more likely to score 1.5 (poor condition), while most other horses present scored 2-2.5 (fair) or even 3 (good), (Scorolli 2020). This again suggests that the energetic demands of lactation are an important driver of body condition. However, a study conducted in the New Forest, England, suggested that habitat use varied according to life history stage: lactating mares changed their patterns of habitat use and would move to areas where higher calorific input was possible, although these might not be part of their accustomed range when non-lactating. Therefore, the relative impacts of parasite loads might be altered by behaviour of the animals.

Moreover, not all studies have reported such high loads in unmanaged populations. A study of >700 feral horses in Louisiana reported an infection rate of 69.2% in 28 sampled individuals but no correlation between FEG and body condition (Cain et al. 2018). A FEG of zero was reported in 10 sampled domestic horses sharing the range with the feral herds, probably because they received regular anthelminthic treatment. Similarly, a study of 293 feral horses in South-Eastern Australia reported a 96.7% infection rate (Harvey et al. 2019), which raised concerns that the feral populations could represent a parasite refugia for domestic horses sharing the range (Barone et al. 2020).

Parasite loads also might be affected by previous exposure to parasites and genetic and early life history affects could influence CS later in life (Putman et al. 1987; Gill 1987). However, whether this is a consequence to the use of habitat, efficiency of forage utilisation or an innate genetic resistance to parasites or a synergy of these effects is not clear. An individual's FEG load could depend~~s~~ more on the host's innate immunity than on their exposure to infection if the hosts are energy saturated and not resource limited (Cressler, Graham, and Day 2015). This implies a negative correlation between FEG and body condition, i.e., good body condition correlates with low FEG when the individual has sufficient energy resources to have efficient immune response to the parasites and *vice versa.* Effective use of habitats, efficient forage utilisation or a synergy of these effects would therefore be more likely lead to energy saturation and consequently to sufficient energetic reserves for the immune response to suppress parasite infection. However, research on the Sable Island feral horse population suggests that the negative correlation between CS and FEG is not due to heritable linked traits (Gold et al. 2019): whilst FEG load capacity was moderately heritable (h^2=0.43, +/- 0.11), CS maintenance was not (h^2=0.04, +/- 0.007). This suggests that the impact of parasite loads on CS could have a strong environmental component. Free-living wild Prezwalski horses in Hungary (Brabender, Zimmermann, and Hampson 2016) and Ukraine (Slivinska et al. 2020) shows similar, but not identical trends in results reported from other feral and wild horse populations. The feral horse populations are descended from escaped or released domestic horses of various breeds/ types whereas the Prezwalski horses are descendants of re-introduced zoo-bred animals, originally obtained from wild caught stock that were for a long time considered to be the last truly 'wild' horses. Evidence from archaeological sites at Botai, Kazakhstan, suggests that Prezwalski horses were domesticated briefly, then 'rewilded' 4.5 thousand years ago (Gaunitz et al. 2018). While there are some shared mitochondrial haplotypes between modern and Prezwalski horses (Kvist and Niskanen 2021), they do have genetic differences from modern feral horses, including a difference in chromosomal numbers (modern horses have 64 chromosomes, Prezwalski horses have 66 chromosomes). This suggests that despite possible inherited differences in parasite load and CS maintenance, Prezwalski and feral horses show the same observed seasonal variation in CS, with the lowest scores in spring, and highest scores in autumn (Brabender, Zimmermann, and Hampson 2016). However, in contrast to the feral horse populations, seasonal fluctuations in CS scores were observed for both stallions and mares. Whether this difference in the CS of Prezwalski mares is due to intrinsic or extrinsic differences in the would require further investigation. A four-year study of 130 Prezwalski horses in the Chernobyl Exclusion Zone, found a similar correlation between seasonality and CS, along with seasonality of FEG that was dependent on herd density.

Overall, this indicates similar, but not identical CS results in feral and wild horse populations.

Free-living Exmoor ponies, or supplementary feeding in winter, and so are subject to natural challenges of parasite loads and of seasonal variation in vegetation quality and abundance, and the increased energy demands of winter weather (Baker 2008). Exmoor ponies are now widely used on rewilding sites throughout the UK and Europe; for example, Knepp rewilding in Sussex (Tree 2019), and Mols rewilding in Denmark (Hagstrup et al. 2020).There are also numerous examples of Exmoors being used in conservation sites: Juliet Rodgers, Chairperson of Moorland Mousie Trust, manages 55 UK conservation grazing sites, home to >200 Exmoor ponies (personal email communication; 22/06/23); Jayne Chapman, Hampshire and Isle of Wight Wildlife Trust, manages numerous Butterfly Conservation and Wildlife Trust sites using Exmoor ponies (personal communication, 28/06/23); Hans Hovens, Chairman Samenwerkingsverband Exmoorpony, reports that there are 602 Exmoor ponies used on conservation sites by 16 organisations in the Netherlands (personal communication, 16/5/23); Bamff Wilding, will host 12 breeding Exmoor ponies to be introduced in January 2024; and Scoraig, Highland hosts >30 Exmoors managed as a rewilded herd (owned by author). On these sites routine anthelmintic treatment or supplementary feeding is usually not permitted. These sites are home to almost all the Exmoor ponies in the Netherlands and to a considerable proportion of the UK Exmoor population. However, there has not been quantification of the possible welfare impacts due to reduced body condition because of gastrointestinal parasite loads when the use of anthelmintics is not permitted.

Improved understanding of the effects of parasite load and seasonality on the CS of Exmoor ponies would assist in monitoring their welfare and informing their selection for suitability and management in conservation and rewilding projects. The aims of this study were to: quantify parasite loads and body condition scores in free-living Exmoor ponies across seasons and years as a proxy for determining whether variation in health is driven more by biotic (FEG) or abiotic (seasonality) extrinsic factors than intrinsic characteristics of individuals (age, sex, reproductive status).

3.3 Materials and methods

3.4 Body condition scoring

Body condition scores (CS) were assessed by estimation of an individual's fat reserves, which are dependent on the balance between energy intake and energy loss (Henneke et.al. 1981). The energy balance which is visually represented by CS depends on many factors, including food intake, parasite load, season, and the age, sex, and reproductive status of each individual pony. A negative energy balance (energy loss greater than energy intake) will result in weight loss and subsequent a reduction in body condition score. A positive energy balance (energy expenditure less than energy intake) will result in increased fat and muscle and subsequent increased body condition. The energy balance depends on many factors, including the availability of food and water, weather (ambient temperature, atmospheric and ground moisture, and wind chill), reproductive activity (e.g., pregnancy, lactation), age, growth stage and health status. Using the approach of Carroll and Huntington (1988), CS was estimated by visual inspection of the entire animal to assess the amount of fat over the ribs, tailhead and quarters, neck, and withers and spine to allow the comparison of ponies with differing amounts of stored body fat, independent of the body size, sex, or age of the pony and also includes physical palpitation of specific body areas as well as visual assessment. Subsequently CS is expressed on a scale from 0 (i.e., very little fat reserve, individual is severely emaciated) to 5 (i.e., extremely high fat reserves, individual is obese). This is quantified in evenly spaced intervals of 0.5 between the lowest and highest scores.

In this study some of the Exmoor ponies were completely unhandled and removing them from their environment and restraining them to allow palpitation was not undertaken as it would have caused them considerable stress. Therefore, a visual inspection and CS score allocation was performed simultaneously but independently with an experienced veterinary surgeon to ensure accuracy. Scores obtained this way, when compared between scorers, resulted in either the same score or had no more than 0.5 discrepancy. The British Horse Society and British Equine Veterinary Association (BEVA) veterinary guidelines (https://www.beva.org.uk/Guidance-and-Resources/Routine-Healthcare/Equine-Obesity#weightmanagement, accessed at 16.43 on 7/11/23) suggest that scores between 2.5 – 3.5 represent ponies in optimal condition according to their age, reproductive status, and the environmental conditions. This condition scoring system is independent of size or conformation of the pony and with experience even young or pregnant animals or those with heavy winter coats can be accurately assessed.

Assessments were carried out twice a year, in spring and autumn, during the three-year study period, resulting in 167 records of CS for 34 individuals.- Due to the demographic structure of the herd, 30 females (including both barren and pregnant mares and immature individuals) were sampled, along with three immature males and one mature stallion. Due to the difficulties in locating free-living individuals with differing habitat usage on a large hectarage (approximately 3000 hectares, >88 hectares/ pony) CS and FEG assessments were not always possible for every individual at each sampling interval. Births and deaths also led to the addition or removal of individual ponies from the study. Therefore, the CS records are not necessarily complete sets for every individual at each sampling interval. Throughout the study all the individual ponies were grazing on the same area of hill ground, except during May to August, when the mature stallion and some selected mares were removed to an adjacent area with similar resource availability to allow breeding management. No individuals received any significant additional supplementary feeding. All individuals in the study therefore had equal access to food, water, and shelter. No individuals received routine anthelmintic treatment. Consequently, the inter- and intra-individual variation in CS can be explained by intrinsic differences in individuals such as their individual identity, age, sex, reproductive status as well as by the extrinsic differences in parasite load and season. Photographic examples of ponies' assessed CS are shown in Appendix 5.

3.5 Faecal egg counts

Routine anthelminthic treatment had not been administered to any individuals in the study for > 5 years. Parasite load was assessed using faecal egg counts (FEG; the number of parasite eggs per gram of faecal matter) for each individual pony at each sampling interval (e.g., spring 2020). The FEG count was calculated using the McMaster technique (Appendix 6), with scores estimated in evenly spaced intervals of 100, ranging from 0-500. A load of 300+ is considered to be a high score in domestic equines. The eggs detected were from strongyle wormed, oval shaped, smooth shelled, approximately 90 µ by 50 µ. Figure 3A is a visual representation of the FEG and CS data.

3.6 Analysis: Modelling influences on body condition

To assess the variables that influenced body condition, a mixed effects generalised linear model (GLMM) approach was used, using the lmer package in the programming environment R. CS records were modelled as a continuous response variable, determined by the explanatory variables age (continuous), season when sampled (categorical: Spring, Autumn), year of sampling (categorical: 2020, 2021, 2022) and FEG (continuous). The model included the interaction effects between the explanatory variables. Since only a single breeding stallion was present during the study sex was not included as an explanatory variable. Since multiple measurements were made on the same individuals, pony ID was included as a random effect. Likelihood ratio tests were used for model selection, retaining the minimal model that significantly explained variation in the response variable.

3.7 Results

Parasites were found to be over dispersed in the population, with the percentage of infected individuals varying from 18.5% to 37.5% across the duration of the study. Spring condition scores for all individuals (range 1.5-3.4) were lower than autumn scores (range 2-4.5) (Table 3.1). The proportion of individuals with parasites detected varied by year and season, with the highest prevalence in spring of 2021 and the lowest in autumn 2022 but the highest parasite load in spring 2020 (Table 3.1). The age range of infected individuals was from one to 27 years, with the yearling sampled in spring 2020 showing the highest overall parasite load (Table 3.2). The three immature males had positive FEG counts recorded, but not the mature stallion. Two individuals were removed from the herd and treated with anthelmintics for welfare reasons during the study due to their low CS, high FEG and an assessment of their general health (Table 3.2). All the recorded low condition scores (CS 1.5) were in the spring and, with one exception, were either young individuals, <3 years old (n=3), or aged individuals >20 years old (n= 2), the exception being a 12-year-old, (n=1) (Table 3.2). Two of the six pregnant or lactating mares in the study were infected, both with a relatively low load of 100 FEG (Table 3.3). An aged mare (27 years; ID: 21029) was in poor condition (CS 1.5) in the spring of 2020 when she gave birth to a healthy foal and, although lactating, improved condition by the autumn (CS 2.5). All pregnant or lactating mares showed increased body condition of 1- 2 condition score points between the spring and autumn assessments (Table 3.3).

Table 3.1: Summary of numbers of ponies sampled, the range of condition scores (CS), faecal egg counts (FEG), and percentage of animals infected by season. N is the number of individuals sampled.

Season	N=	Age	Sex	FEG	CS
Spring 2020	1	26	F	100	1.5
	1	7	F	100	2.5
	1	4	F	100	2
	1	3	M	100	2
	1	2	F	300	2
	1	2	F	200	2
	1	1	F	500*	1.5
total	**7**				
Autumn 2020	1	26	F	100	2.5
	1	8	F	100	3
	1	7	F	100	3.5
	1	4	F	100	4
	1	3	M	200	3.5
	1	2	F	100	3.5
total	**6**				
Spring 2021	1	27	F	100	2.5
	1	11	F	100	2
	1	9	F	100	2
	2	9	F	200	1.5 and 1.5
	1	5	F	100	3
	1	4	M	100	2
	1	3	F	100	2
	1	3	M	100	2
	1	2	M	200	2
	2	2	F	200	2 and 1.5
total	**12**				
Autumn 2021	Not available				
Spring 2022	1	27	F	100	1.5
	1	12	F	100	1.5
	1	6	F	100	2
	1	5	M	100	3.5
	2	4	F	100	2
	1	2	F	300*	2
total	**7**				
Autumn 2022	1	27	F	100	2.5
	1	12	F	100	4
	1	6	F	100	3.5
	1	5	M	100	3.5
	1	4	F	200	3
total	**5**				
Overall total	**37**				

Table 3.2 Ponies infected with parasite loads shown by season, age, sex, faecal egg count and body condition score. An asterisk indicates individuals removed from the herd and given anthelmintic treatment for welfare reasons.

Season	N=	CS/ FEG	Range of scores	Infected %
Spring 2020	25	CS	1.5 - 3.5	
	21	FEG	0 - 500	30.4
Autumn 2020	25	CS	2.5 - 4	
	25	FEG	0 - 200	22.2
Spring 2021	30	CS	1.5 - 3.5	
	30	FEG	0 - 200	37.5
Autumn 2021	30	CS	2 - 4.5	
	30	FEG	Not available	Not available
Spring 2022	24	CS	1.5 – 3.5	
	23	FEG	0 - 300	25
Autumn 2022	23	CS	2.5 – 4.5	
	23	FEG	0 - 200	18.5

Table 3.3 Pregnant and lactating mares infected with a parasite load shown by identity (ID), year, age, Reproductive status: in-foal (IF) or lactating (L), condition score (CS) and parasite load (FEG)

ID	Year	Age	IF/L	Spring CS	Spring FEG	Autumn CS	Autumn FEG
21029	2020	27	L	1.5	100	2.5	100
21134	2020	4	IF	2	100	4	100
21134	2021	5	L	3	100	n/a	n/a
21091	2020	14	IF	2	0	3.5	0
21091	2021	15	L	3	0	4	n/a
21109	2021	12	IF	2	0	4	n/a
21109	2022	13	L	2	0	3.5	0
21125	2022	10	IF	2.5	0	4	0
78168	2022	12	IF	2	0	4	0

The final GLMM model included an interaction between age and season **(LRT test statistic, df, p value)** and a main effect of FEG **(LRT test statistic, df, p value)** on CS scores. CS showed an overall decrease with increasing FEG scores (SLOPE), which was observed in both seasons, although CS values were consistently higher in the autumn than the spring (Figure 3.1). Maximum parasite loads were also lower in the autumn (0-200) compared to the spring (0-500) (Figure 3.2).

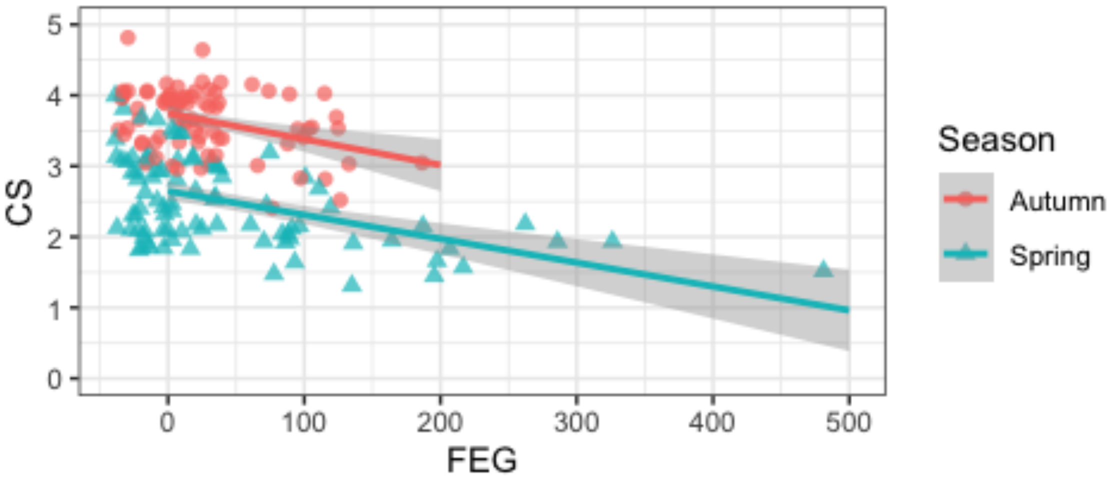


Figure 3.1 Observed body condition scores (CS) and faecal egg counts (FEG, eggs/g) by season. Points have been jittered to show them more clearly. Note that there is decreasing CS with FEG in both seasons, but the CS scores are consistently higher in the autumn than in the spring.

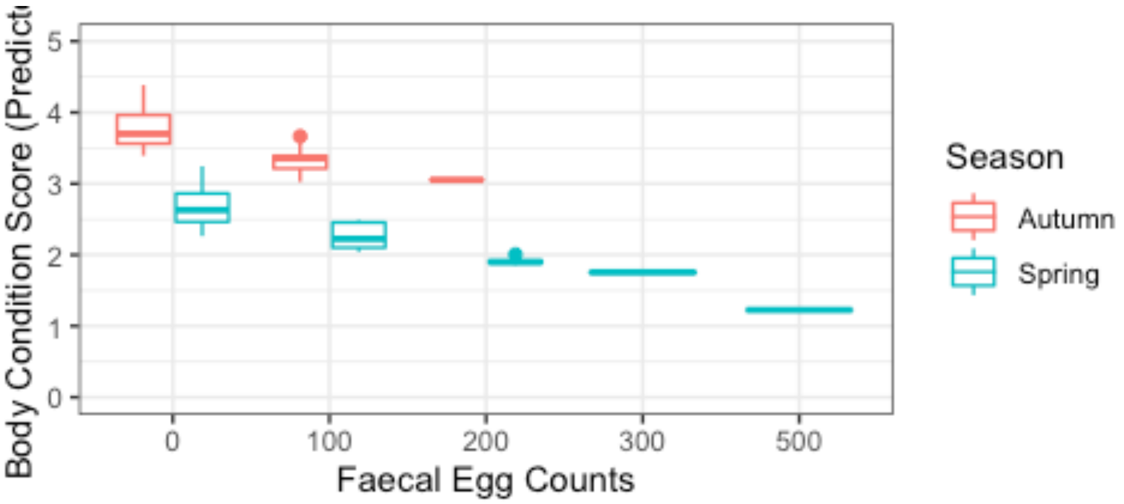


Figure 3.2 Predicted body condition score (CS) against FEG (faecal eggs/g) by season. The model predicts a similar decrease in CS with increasing FEG in spring and autumn for low egg counts (<200 eggs/g), higher egg counts in the spring, and higher CS in autumn irrespective of FEG.

3.8 Discussion

Overall, body condition scores of free-ranging Exmoor ponies decreased in relation to parasite loads in both spring and autumn but both prevalence and intensity of infections were consistently higher in the spring when body condition scores tended to be lower. The range of spring condition scores (1.5 – 3.5) for all individuals was always lower than the range of autumn scores (2 -4.5), indicating that all individuals improved in condition from a low score in the spring to a peak of condition score in the autumn, but the specific effects of season were influenced by the age of the individuals, with the youngest and oldest individuals showing the highest parasite loads and lowest condition scores. The percentage of infected individuals varied from 18.5% to 37.5% during the study period but most individuals showed relatively mild infections (<300 FEG), suggesting that parasites were not a major driver of fitness in this population in the sampling years. These results are broadly in concordance with those previously published from investigations into CS and parasite load in other feral and wild horse populations (Scorolli 2020; Jenkins et al. 2020; Slivinska et al. 2020; Debeffe et al. 2016; Brabender, Zimmermann, and Hampson 2016; Gill 1987; Putman et al. 1987). Seasonal effects on body condition and parasite loads have been widely reported, possibly due to reduced resource availability in winter being common in in temperate regions. Cressler, Graham, and Day (2015) suggested that reduced CS could be related to lowered parasite resistance due to a negative energy balance consequent to resource limitation during the winter. In contrast, study on feral horses in Louisiana, where seasons are less extreme than in Scotland, reported no seasonal resource limitation, and found no significant relationship between FEG and body condition, suggesting that access to sufficient resources provided energy for immune system support throughout the year (Cain et al. 2018). The individuals in this study, located in the Scottish Highlands, were resource limited in winter, potentially reducing energy available for immune support, in turn suggesting that the finding of higher FEG in the spring might potentially be because of reduced immune response during the winter season.

A study in southern England observed deterioration in CS in all New Forest ponies from December until late spring, when improvement in condition of non-lactating individuals begins, with the highest CS being attained by all ponies in autumn (Gill 1987). As for my study, the CS of lactating mares also was observed to improve between the spring and autumn, as the energetic demands on the mare are reduced as the foal increases in independent. The oldest lactating mare in the study (27 years old) produced a healthy foal in the spring of 2020 despite poor body condition and the presence of parasites across all sampling seasons, which could be due worn teeth) limiting her utilisation of reduced quality winter forage or an age-related decline in immune function. Mares may change their patterns of habitat use to improve their energetic intake during lactation (Gill 1987), but these potential changes were not quantified during this study. In my study, there was not even spatial distribution of the ponies over the available area since they aggregate in different social groups and move around the overall area according to preferred habitats within the area (e.g., resource preferences between grassy lawns, bog, heather moorland, and seashore with access to seaweed) and in response to weather and seasonal conditions (e.g., shelter from prevailing winds, access to running water in freezing or drought conditions, seasonally driven variable changes in food resources). Continuation of this study, using Global Positioning System (GPS) collars, could usefully investigate possible seasonal or life history stage changes in habitat use and improve understanding of the relationship between habitat and CS.

Additionally, Density dependent effects on FEG as well as overdispersion of parasite prevalence and intensity have been found in other studies on feral horse populations but parasite loads were generally lower than in other studies (Debeffe et al. 2016; Rubenstein and Hohmann 1989; Jenkins et al. 2020), which could suggest that Exmoors from Scoraig are not exposed to parasites as frequently as in some other habitats, possibly because the area that they roam is sufficiently large that population densities are lower. Whether the relatively lower loads reported in this study are due to different habitats, habitat usage, density dependence or due to Exmoor ponies having a higher resistance to parasites when compared to the feral populations cannot be ascertained from the results of this study, but further investigation might offer elucidation.

A previous study on Sable Island horses (Gold et al. 2019) suggested moderate heritability of parasite loads (h^2 =0.43, +/- 0.11). If this is also true for Exmoors and the low parasite loads are due to anthelminthic resistance rather than differences in exposure, it could be a trait that breeders could consider as a target for selective breeding. Exmoors' possible natural resistance to parasite loads would also indicate their suitability for inclusion in restoration and rewilding projects where anthelminthic use is either restricted or not permitted.

3.9 Acknowledgements

Barbara Mable created the mixed effects generalised linear model (GLMM). Vivien Taylor, MRCVS, verified the CS scores.

Chapter 4: Combining pedigree and molecular data to inform conservation management of Exmoor ponies

4.1 Abstract

Exmoor ponies, an endangered rare breed, underwent a genetic bottleneck in the 1940s to <50 individuals. Subsequent efforts to preserve the breed have been based upon sometimes incomplete or inaccurate pedigree records but molecular data could provide. improved accuracy in targeted breeding for conservation. This study combined stud book and historic records with mitochondrial DNA (mtDNA) and whole genome sequencing (WGS) to: 1) verify matrilines based on observational pedigrees using mtDNA control region sequences; 2) determine relationships between individuals using WGS; 3) estimate inbreeding based on runs of homozygosity (ROH) from WGS; 4) investigate deleterious allele frequencies in relation to inbreeding; and 5) inform future breeding strategies. mtDNA haplotype networks revealed overall congruence with recorded matrilines, with uneven representation of the haplotypes within the population but also identified some inaccuracies in recording maternal lineages. Comparison of FROH (10 Mb) values with 23 other breeds showed that Exmoors have the highest levels of recent inbreeding), but there was varying intensity of inbreeding between herds of Exmoors. The proportion of loss of function to synonymous derived alleles declined significantly with increasing 10Mb F_{ROH} values, possibly indicating purging. These results improve stud book record accuracy and understanding of inbreeding and relatedness.

4.2 Introduction

The current anthropogenically driven biodiversity and climate crises are driving species extinction rates several orders of magnitude higher than the natural norm, so that about 25% of wildlife species are currently at risk of extinction (Svenning 2020). This loss of biodiversity will alter ecosystems such that their regulatory functions could be compromised and the goods and services they provide could be diminished (Cardinale et al. 2012). Species that perform the function of ecosystem engineers, by imposing direct or indirect regulatory control of the resources accessible to other species by alteration of the physical state of the biotic or abiotic materials in their environment, thereby modifying and controlling their habitats, are crucial in maintaining the functioning of those habitats (Jones, Lawton, and Shachak 1994). Losing these species can therefore have a significant impact on the overall biodiversity of an ecosystem, affecting the health and functioning of the entire ecosystem. Thus, the conservation of healthy populations of these species is important not only for the maintenance of biodiversity of those specific populations, but also for the knock-on impacts on the entire habitat.

Maintaining high species intra-population genetic diversity improves the potential for population evolvability and adaption to future environmental change, reducing extinction risk (Kahilainen, Puurtinen, and Kotiaho 2014). Knowledge of the genetic history of a population, which can inform the maintenance of its genetic diversity should therefore be a priority in programs aimed at conservation of rare or endangered populations. One approach to understanding the genetic history of a population is using mitochondrial DNA (mtDNA), which is maternally inherited, nonrecombining, exhibits rapid evolution, and has extensive intraspecific polymorphisms and is therefore very suitable for tracing the history of maternal line diversity within a population (Avise et al. 1987). Haplotype networks are a visual representation of the relationships among different mtDNA haplotypes, illustrating their evolutionary connections, with the length of the connections representing the genetic distance between them. mtDNA studies have been very widely used in both human studies where the origins of populations were under investigation (Helgason et al. 2000) and animal studies to inform conservation priorities (Antunes et al. 2007; Mucci et al. 2010; Rosauer et al. 2016; Zink et al. 2010).

Genetic rescue of populations that have experienced severe bottlenecks has often relied on assessment of remaining haplotype diversity in wild populations, with management aimed at maintaining existing diversity, supplementing diversity from geographically distant populations or with extant zoo populations, which often have preserved haplotypes that are rare or absent in wild populations. For example, a study by Wójcik et al. (2009) investigated the mtDNA of 87 endangered European bison, *Bison bonasus,* and found only three distinct haplotypes. They concluded that the low mtDNA variability was in concordance with the theoretical expectations for a species that had recently undergone a severe population bottleneck. Results from the study informed management strategy for the preservation of the haplotypes present in the extant population.

Genetic rescue can be based either on supplementing the breeding population by the translocation of individuals from another, unrelated population to improve fitness through heterosis, or by adaptive evolution, phenotypic improvement through selection through selection (Whiteley et al. 2015). In a similar study of 77 Indian tigers, *Panthera tigris tigris*, Sharma et al. (2009) found that Northern tiger populations exhibited two unique haplotypes, suggesting genetic isolation. However, an extinct Sariska population and an extant Ranthambore population were sufficiently genetically similar, that they suggested that the latter could provide a source for reintroduction into the former.

Conservation strategies also benefit from establishment of historical origins of rare or ancient lineages. Studies comparing ancient and modern horse genomes reveal that considerable haplotype variation exists in the modern domestic equine mitogenome, suggesting multiple spatial and temporal introgressions of mtDNA (Cieslak et al. 2010). A study of 207 ancient and 1754 modern horses sampled from across the Eurasian continent found 87 ancient haplotypes originating from the Pleistocene to Mediaeval periods, although only 39 were found in modern breeds (Cieslak et al. 2010). This indicates that this haplotype diversity is of ancestral origins and suggests that post-domestication breeding practices have reduced mtDNA diversity (Cieslak et al. 2010). The genetic relationships between major geographical haplotype clusters are not yet clear, but an investigation of Iberian equine mitogenomes found that although they predominantly came from Arabian and Barb horse descent, thus were of North African origins, the Northern Iberian breeds, whose phenotype resembles Exmoor ponies, showed introgression from these British pony types (Royo et al. 2005; Lira et al. 2010).

An hypothesis concerning the origin of modern horses suggests that waves of migration of early horses across the Bering Straits land bridge circa 37 thousand years ago led to them developing into four distinct primitive ancestral types, varying according to their geographical destination: the proto- Arab, adapted to North African and Middle Eastern conditions; the Steppe horse, adapted to Central Eurasian conditions, the Ancestral cold blood, adapted to Central and Northern European conditions; and the Universal Pony type that was found from the Steppes to the Atlantic seaboard (Schafer 1981). Schafer suggested that modern breeds were developed from mixtures of these ancestral types. He also stated that the modern Exmoor pony closely resembled the Universal Pony type and was likely its most direct descendant, giving support to the idea that Exmoors are an ancient, un-mixed breed.

Of the three mtDNA haplotypes found in the extant Prezwalski horse population, two are distinct from, but one is similar to domestic horse populations (Goto et al. 2011; Librado et al. 2016). This would support recorded, but sometimes disputed, evidence that the extant population, descended from 12 foundation animals, included domestic or partly domestic horses as well as animals from the remaining wild population (Bokonyi 1987; Der Sarkissian et al. 2015). When prioritising conservation of Prezwalski horses, this improved information about the likely origins of particular founders can improve targeted breeding programs.

In contrast with the high diversity of mtDNA in equines, there is little significant diversity in their Y- chromosomal sequences (Wallner et al. 2013; Cardinali et al. 2022). Post-domestication horse breeding practice usually uses a harem-based breeding system, similar to thar found in the wild, but with a single stallion, rather than potentially multiple stallions, covering a large number of mares, (Jaworska et al. 2020; Madosky 2011) which could explain at least part of this lack of diversity (Ellegren 2002). A study investigating polymorphisms in noncoding Y-chromosome sequence among 52 male horses of 15 different breeds did not identify a single segregating site (Lindgren et al. 2004). Nevertheless, Bozlak et al. (2023) found that the phylogenetic placement of 163 ancient Y-chromosome haplotypes indicates that most of the extant Y-chromosomal variation appeared during the last four thousand years, i.e. post-domestication. These observations are consistent with a strong sex-bias in the domestication process, with few stallions contributing genetically to the domestic horse. Evaluation of the potential variation in the Exmoor pony Y- chromosome became of particular interest in March 2023 when the Exmoor Pony Society launched its Gene Bank Campaign with the aim of storing frozen semen from 25 stallions, 50 doses from each individual, representing as much of the breed’s genetic variation as possible, to be held in long-term storage at two separate locations, as ‘catastrophe insurance’ (https://exmoorponysociety.org.uk/the-exmoor-pony-society-gene-bank-campaign/). Since fewer than 15% (600 individuals) from the total Exmoor pony population is actively breeding, they are more vulnerable than in populations where a higher proportion of the population can breed, so this type of additional protection is particularly important (https://exmoorponysociety.org.uk/the-exmoor-pony-society-gene-bank-campaign/). Prior to the launch of this campaign, semen from six Exmoor stallions was collected and stored in the National Livestock Gene Bank but only three of these have sufficient good quality doses to be considered viable, as some stallions appeared to yield low quality semen, or semen that did not freeze satisfactorily despite their being successfully fertile when used for natural service (personal email communication with Sue Baker, Chair of the Exmoor Pony Society Scientific Panel). Whole genome-based analysis of Y-chromosomes could thus be beneficial to enable prioritisation of semen storage.

Where whole genome sequences (WGS) are available, single nucleotide polymorphism (SNP)-based approaches can be used to assess genetic diversity, relatedness among individuals, quantify levels of inbreeding and assess accumulation of deleterious mutations within populations, as well as quantifying patterns of differentiation between populations. Due to large number of genetic markers accessible through WGS population genetic inferences have become more accurate. For example, measuring inbreeding in the absence of very deep pedigree data has become possible based on Runs of Homozygosity (ROHs), which are contiguous stretches of the genome where an individual inherits identical copies of DNA from both parents due to recent shared ancestry (Ceballos et al. 2018). ROH can also provide information about inbreeding over different timescales since the lengths of ROH are proportional to the time to the most recent common ancestor, as recombination breaks up linkage over time. Thus, longer ROH segments suggest more recent shared ancestry than shorter segments.

For example, Signer-Hasler et al. (2023) used ROH to investigate relatedness and inbreeding in 18 European Alpine cattle breeds compared to more widespread commercial cattle, discovering that the proportion of homozygous animals was higher in all studied animals of local breeds than in commercial cattle. Higher average inbreeding assessed based on runs of homozygosity (F_{ROH}) and higher average intra-breed genomic relatedness were found in the local Alpine breeds derived from small founder populations. ROH islands near genes related to thermoregulation, colour, growth rate and size suggested local adaptation to the alpine environment during the evolution of local Alpine cattle breeds. Understanding these local adaptations is important in breeding stock able to thrive in specific, in this instance alpine, conditions. Rare breeds with small, local populations, often survive due to these adaptations to environments where other less hardy breeds do not thrive. Arguably this type of investigation of the Exmoor pony genome could offer insights into their observed survival adaptations, as most are still bred in free-living herds. Quinn et al. (2023) investigated 59 individual Cape buffalo ranges hey found that Cape buffalo have high average heterozygosity overall (0.40) with two sub-populations having significantly lower than average heterozygosity level (0.33 and 0.29), similar to that found in domesticated water buffalo (0.29). These lower levels could be due to recent inbreeding or a small ancient founder population. These types of insight into population genetic history could be usefully applied to the Exmoor pony population.

All individuals carry deleterious mutations within their genomes which may reduce that individual's fitness (Frankham, Briscoe, and Ballou 2002; Bosse et al. 2019). This mutation load will vary according to the background mutation rate, demographic history, and selection applied within those populations (Frankham, Briscoe, and Ballou 2002; Bosse et al. 2019). Most of these mutations are likely to be recessive, so therefore only expressed in the homozygous state, which is more frequently observed in inbred populations, and is known as the genetic load (Crow 1970; Feldman and Crow 1970; Mukai et al. 1972). In inbred populations, inheritance of regions of identical copies of genetic material from related parents, ROHs, may potentially cause negative effects due to the presence of homozygous deleterious alleles (Curik, Ferenčaković, and Sölkner 2014). The decline in fitness observed in inbred progeny, due to accumulation of homozygous deleterious alleles, is known as "inbreeding depression" (Keller and Waller 2002). The inbreeding load may, however, be reduced by purging, increased purifying selection facilitated by a long history of inbreeding (Hedrick and Garcia-Dorado 2016). Purging occurs as inbreeding increases the frequency of homozygotes, so that their effects are no longer masked, but are exposed to selection, so that those causing severely detrimental effects (for example, loss of function alleles), are purged from the population (Hedrick and Garcia-Dorado 2016). Less seriously deleterious alleles may also be purged, but may, if their effects do not severely compromise fitness, accumulate within an inbred population (Hedrick and Garcia-Dorado 2016; Bosse et al. 2019). Purging can be deliberately induced as a conservation method by subdividing a population into distinct, isolated sub-populations, although genetic drift can, over the long term, reduce the rate of purging of mildly deleterious alleles or even fix them in the population (Hedrick and Garcia-Dorado 2016). Population bottlenecks are likely to reduce genetic diversity, and thus could increase the mutational load (Cruz, Vilà, and Webster 2008). Species domestication is often associated with population bottlenecks, which in turn tend to result in increased genetic load, with Marsden et al. (2016) reporting that the ratio of amino acids changing heterozygosity to silent heterozygosity (variants presumed to have no effect) was higher in domestic dogs than in their ancestors, wild wolves. This finding suggests reduced ability of purifying selection to remove weakly deleterious variants is reduced post-bottleneck (Marsden et al. 2016).

Understanding of the frequency and distribution of deleterious alleles in a population could be utilised in breeding programs to avoid the expression of these traits and in planning to avoid future problems caused by high genetic load (Bosse et al. 2019). An example of the importance of this is illustrated in Fell ponies, a rare breed native to the north of England, and believed to originate from the crossing of foreign stallions imported by the Romans to serve along Hadrian's and the Antonine Walls with local, native, Exmoor-type mares (Richardson 1990). It was noticed in the early 2000s that ~10% of Fell foals, although apparently normal at birth, developed severe progressive anaemia, multiple infections unresponsive to treatment, and died or were euthanised at <3 months old (Fox-Clipsham et al. 2011). On investigation a lethal Mendelian recessive disease, Foal Immunodeficiency Syndrome (FIS), was found to be the cause (Fox-Clipsham et al. 2011). A mutation on chromosome ECA26 of the sodium/myo-inositol cotransporter gene (SLC5A3), which regulates osmotic stress during early embryonic development, had undergone an amino acid substitution, altering the function of SLC5A3, leading to later failure of the immune system (Fox-Clipsham et al. 2011). The subsequent development of a diagnostic DNA test to identify symptom-free carriers allowed breeding programs aimed at the elimination of FIS from the population.

A further consideration is to offer guidance to breeders considering the introduction of new breeding stock into their herds. To be effective, new introductions need to be carefully planned. An example of the care needed in introducing new stock comes from a study of Eastern Black rhinos, (*Diceros bicornis michaeli*), which found that natural dispersal could be more effective than re-location for population rescue, because although both translocated and naturally dispersed individuals showed lower inbreeding compared to closed populations, the frequency of deleterious mutations was higher in the offspring of the translocated individuals (Mellya et al. 2023). This could lead to inbreeding depression if these carriers of deleterious alleles subsequently became inbred.

Another example of why understanding of genomics can assist in breeding programs is when information about the heritability of selected traits can be utilised to potentially improve the welfare of populations in the future. Gastrointestinal parasites can have a significant impact on the hosts' development, condition, health, reproduction, and longevity (Debeffe et al. 2016). Studies of feral horse populations in the USA report a negative correlation between gastrointestinal parasite load and lowered body condition scores (Rubenstein and Hohmann 1989; Rudman and Keiper 1991; Debeffe et al. 2016; Jenkins et al. 2020). In Sable Island feral horse populations, parasite loads have been demonstrated to show moderate heritability (h2 =0.43, +/-0.11) (Gold et al. 2019), suggesting that breeding for resistance could be possible. This suggests that an investigation into the relationship between parasite load and inbreeding in Exmoor ponies could be relevant, since many Exmoors are kept on conservation or rewilding sites where the use of anthelminthic drugs is either restricted or prohibited and breeders could investigate prioritising individuals likely to carry low parasite loads.

4.3 Why conserve Exmoor ponies?

The Rare Breeds Survival Trust (RBST) are an oversight organisation for non-zoo bred rare, mostly domestic breed animal populations in the UK. They publish a watch list covering equine, cattle, sheep, pig, and poultry breeds whose populations are numerically small and/or declining. Effective population size and inbreeding calculations, usually based on mean kinship from observational pedigrees, are used to assess a breed's conservation status. The data used in producing this watchlist is taken from records kept by the Department for Environment Food and Rural Affairs (DEFRA) and breed society records. Breeds are assigned to the categories' 'priority' or 'at risk', according to the assessment of the population status (https://www.rbst.org.uk/watchlist-overview, accessed 9.45am, 16/01/23). Exmoor ponies are currently classified by the RBST as a 'priority' equine breed.

There are many claims that Exmoors are likely to be closely related to the ancient wild horse found in NW Europe after the last Ice Age (Baker 2008; Hovens 2014; Dent 1987; Hulme 1980), based on: 1) their external phenotype, closely resembling that of the primitive equids depicted in cave paintings (BernÁldez-Sánchez and García-Viñas 2019); 2) their anatomical and morphological features, resembling ancient remains (Speed and Speed 1977; Hovens 2014); 3) their apparent retention of 'primitive' characteristics (Goodwin, Levine, and McGreevy 2008); 4) written evidence of their distinct characteristics, resulting in their being pure-bred in a discrete population over a long period at a time when many breeds were being deliberately cross-bred to 'improve' them (Sydney 1893; Hayes 1897); and 5) their adaptation to survive in harsh natural environments (Baker 2008; Hovens 2014; Speed and Speed 1977). Schafer (1981) hypothesised that modern horses are the descendants of four types of primitive equine and that the Exmoor pony closely resembles the primitive type he calls 'the universal pony'. As a rare breed, believed to retain ancient characteristics, and since most Exmoors are bred in free-living herds under continual environmental selection pressure, they are of special conservation interest.

4.4 Exmoor pony Stud Book records

More recently, the original herd of pure-bred Exmoor ponies on Exmoor was the Anchor herd, whose records date back to the 17th centaury. Herds 1, 12 and 23 were the other principal herds of pure-bred ponies, founded in the late 19th and early 20th centauries with stock originally from the Anchor herd (Baker 2008; Dent 1962; Hayes 1897; Sydney 1893; Society 1980; Society). Although some matrilines have been lost since the foundation of the herds, many have direct matrilineal descendants extant. Of the matrilineal lines recorded in the current stud book, nine originate from and are still bred (although not exclusively) within the Anchor herd, based on Exmoor; six matrilineal lines originating from Herd 1, which was based on Exmoor but closed in 1960, are maintained through breeding stock distributed to other herds; two matrilines are extant from Herd 12, which is still based on Exmoor, although these lines are bred in other UK herds; three lines from Herd 23, still based on Exmoor, exist in the UK, with others currently maintained in European herds; and seven more matrilines, from herds originally based on Exmoor but now closed, have been dispersed to other existing breeding herds throughout the UK and Europe. These are the matrilines with records pre-dating the 1980 Exmoor Pony Society Stud Book. An additional matriline is extant, descended from a mare ‘taken on inspection’ though probably bred on Exmoor, whose ancestral origins are not recorded. The founder of this matriline was accepted for registration, after EPS inspection of her phenotype (EPS Stud Book, 1980). The EPS Stud Book is now closed: only foals born to fully registered and approved parents are eligible in the main section of the Stud Book.

To be eligible for registration in the EPS Stud Book, foals must be descended from fully registered parents, their parentage must be verified by a DNA test; the sire should hold a licence showing him to be approved for breeding by the EPS; and the foal must be approved for registration by two EPS approved Inspectors who perform an assessment of whether its external phenotype conforms to the published Breed Standard (https://exmoorponysociety.org.uk/about-breeding/).

In 1980 the Exmoor Pony Society (EPS) published the third edition of its Stud Book which recorded the registration details, including pedigrees, of all ponies born and accepted for registration since the resumption of pony inspections after the Second World War (WW2), from 1945 onwards. This Stud Book was intended to be comprehensive and include all registered Exmoor ponies in one edition. Previously ponies had been recorded in the EPS Stud Book, the National Pony Society (NPS) Stud Book and in various private owners' stud books. The Exmoor Pony Society now also maintain an online stud book, managed since 2021 by Grassroots, the registration facility offered to breed societies by the Rare Breeds Survival Trust.

Breeders have hitherto relied on Stud Book records to plan their breeding programs, to select ponies as outcrosses for their herds to reduce inbreeding, or to choose to breed from ponies from matrilines that appear to be under-represented in the Stud Book to increase genetic diversity and to reduce the risk of further loss of matrilines. Breeders have also selected their breeding stock based on its herd of origin, believing that stock from specific herds is either: 1) unrelated; or 2) related and exhibiting a desired phenotype such that through deliberate inbreeding they attempt to fix a specific phenotype within their herd. These strategies have always relied on the accuracy of Stud Book records.

4.5 Why use molecular methods?

Dell et al. (2021) states that while pedigrees alone are no longer adequate to formulate breed management programs, where multi-generational pedigrees are available, they remain a useful tool in formulating breed management programs, where the aim is controlling the rate of increase in inbreeding. However, the reliability of pedigree-based analyses and of advisory breeding programs based solely on stud book pedigrees, depends on the accuracy of those stud book records. Studies using mtDNA suggest that understanding of the genetic history of a population can be improved using comparison of DNA sequences (Librado et al. 2016; Goto et al. 2011; Sharma et al. 2009; Wójcik et al. 2009). This would suggest that using a combination of pedigree and molecular analysis might offer the most accurate and therefore optimal information for breeders aiming to devise long-term conservation management programs. Moreover, the feasibility of now analysing whole genome sequence data from different herds could revolutionise perspectives on the impacts of past management strategies. Investigation into the genetic diversity, inbreeding and genetic load as shown in other studies into rare populations (Signer-Hasler et al. 2023; Quinn et al. 2023), would offer insights into the current and historic inbreeding within the population, informative for breeders planning management strategies.

4.6 Aims

The overall aims of this study were to investigate whether genetic data could be used to inform management of Exmoor pony breeding strategies. This study combined stud book and historic records with mitochondrial DNA (mtDNA) and whole genome sequencing (WGS) to: 1) verify matrilines based on observational pedigrees using mtDNA control region sequences; 2) determine relationships between individuals using WGS; 3) estimate inbreeding based on runs of homozygosity (ROH) from WGS; 4) investigate deleterious allele frequencies in relation to inbreeding; 5) inform future breeding strategies; 6) compare inbreeding at various timescales (based on runs of homozygosity) of Exmoors compared to other herds and determine whether there are differences between extant Exmoor herds; 7) determine whether there are differences in genetic load in relation to levels of inbreeding; and 8) determine whether individual parasite loads are related to levels of inbreeding of individuals.

4.7 Methods

Note: I performed the sampling, analysed the pedigree and interpreted all results in the context of the pedigree and my knowledge about the biology of the horses. Although the genetic data were generated by others (acknowledged in each section) it was my interpretation that is critical for informing management of the ponies.

Pedigree Analysis

EPS Stud Book records were accessed via The Exmoor Pony Society Stud Book, 3rd Edition, 1980, and the online Stud Book (https://breeds.grassroots.co.uk/Home), The National Pony Society Stud Book 1913-47, and breeders' personal records. These records were accessed to investigate which females breeding post-World War II (WW2), when the Exmoor pony population underwent a genetic bottleneck, have extant direct female matrilinear descendants. These female founders of the current population and the current location within the UK of their extant female matrilinear descendants was identified. This information was then used in the selection of ponies for DNA sequencing.

Sampling

To obtain a comprehensive overview of the genetic diversity within the current Exmoor pony population in the UK, hair samples, used for DNA extraction, were obtained from all the matrilineal founder lines represented in the extant breeding mare population, (and from males representing the founder patrilines when possible). Samples were selected according to Stud Book pedigrees to represent 23 of the 24 matrilineal lines of descent from the post-WW2 founder population present in the UK (no samples were available from one matrilineal line with <5 live females). When possible, samples were collected from multiple individuals representing each matrilineal line to improve the accuracy of the verification of the maternal haplotypes present in each Stud Book represented matrilineal founder line. Individuals from the same Stud Book identified founder line should share mtDNA haplotypes. The presence of multiple mtDNA haplotypes from a founder line would indicate that multiple females, not the single founder identified in the Stud Book, were ancestors of the extant individuals.

Samples were collected, when possible, by plucking approximately 20 hairs with follicles attached from the tails or manes of the sampled individuals, the sampler changing gloves or cleaning hands between each collection to avoid cross-contamination. Samples were stored in labelled paper envelopes or sealed plastic bags until extraction of DNA from the hair follicles. Some blood and hair samples from deceased ponies were made available by last three letters of a registration number are the individual's unique identifier within that herd, any numbers prefixing those identify the herd into which that individual is registered (Table 4.1). However, ponies from the Anchor herd, where an anchor symbol is used instead of a herd identification number were recorded in the Stud Book as 000, for clarity. I allocated them the herd number 900; therefore, the founder pony 000041, was individual 41 from the Anchor herd; her sampled descendent from the current Anchor herd has sample ID 900600 and is therefore Anchor herd pony, individual 600. The founder matriline IDs correspond to the herds of origin of the founder mares. The pony sampled IDs correspond to the herds the sampled ponies were born and registered into. The pony sampled ID and founder matriline ID will often be from different herds as they are multiple generations apart. The founder herds sold stock to other breeders who registered the progeny into their own herds, and foundation Herd 1 was closed in 1960.

The foundation mares of the extant Exmoor pony population were identified from Stud Book records, and their Stud Book identification numbers and names were recorded. Most ponies were identified by a herd number, a unique number allocated to their breeder by the EPS to identify all the ponies born into that herd, followed by that pony's unique individual number within that herd; e.g., the founder ID 1020 indicates that the pony originated in Herd 1, and that her individual identity within the herd was 20. The details of the samples collected, their sire and dam lines as recorded in the EPS Stud Book, date of whole genome sampling are shown in (Appendix 1, Table A1.1).

Founder representation

Founder representation was calculated using EPS Stud Book records, to show the number of live females and live potentially breeding males whose pedigrees can be traced to specific founders. Males known to be castrates or not approved for breeding were excluded.

Effective breeding population

Effective breeding population (*Ne*) values were calculated from Exmoor Pony Stud Book records from 1972- 2022. Ne was calculated based on the formula $Ne = 4NmNf/ (Nm + Nf)$, where Nm is the number of males and Nf is the number of females. Information was obtained from the Grassroots online registration system (https://breeds.grassroots.co.uk/Home).

Table 4.1 Founder matrilinear lines with extant female descendants sampled during the study: Extant sampled ponies are shown by founder line descent, mtDNA haplotype and the date of WGS where applicable. The notes are additional details relevant to specific founder lines. Females are shown in black, males in red.

Founder Matriline ID	Founder name	Pony sampled ID	Pony sampled name	mtDNA haplotype	WGS date	Notes
000041	Mayfly	900600	A. Hoki	7		
000049	Heatherglow	458022	D.Gamora	7		Only 3 females extant
		458028	D.Mantis	7	Oct 22	
000050	Milkbar	21098	Sgribhainn	7	Mar 21	
		21137	Finse	7		
		21138	Stob Ghabhar	7		
		276016	C.Kaldens	7		
000051	Mopsy	900588	A. Harlequin	7		
		21131	Badnabae	7	Mar 21	
		107013	M.Knave	7	Oct 22	
		276021	C.Luck Penny	7	Mar 21	
		276023	C.An Teallach	7	Oct 22	
000052	Margarite	900717	A. Orchid	7		
		102004	K. Coritani	7	Oct 22	
000054	Mercury	900241	Ruby	7		
		900658	A. Lapis Lazuli	7		
		900694	A. Nugget 2	7		
		900741	A.Peridot	7		
000002	Marmoset	900234	Rabbit	7		
		900547	A. Galliano	7		
000003	Mushroom	237009	Nutina	7	Mar 21	
000005	Magpie	519004	B. Braveheart	7	Oct 22	
		519005	B. Oozlum	7	Oct 22	
001011	Old Show	Not sampled				<15 extant, mostly non-UK
001020		012157	H. Faberge	7	Mar 21	
		021029	Feith an Fheoir	7	Sept 20	
		021034	Leac na Saidh	7		
		021091	MeallnanPeitherean	7		
		021119	Hoawa	7		
		021134	Balnakiel	7	Mar 21	
		021135	Floyen	7		
		021136	Linnea	7		
		021141	Meabh Cuilag	7	Oct 22	
		049125	C.Rhum	7	Mar 21	
		078150	D.Tree Pipit	7	Mar 21	
		078173	D.Parakeet	7		
001028	Brackenbelle	078170	D.Teal	5	Oct 22	
		078181	D.Black Grouse	5	Oct 22	
001030	Hawkridge Belle	021092	Corrihallaidh	7	Sept 20	
		021109	Fannich	7	Sept 20	
001031		235016	C.Pearl	7	Mar 21	
		479022	MG. Carnelian	7	Mar 21	

001035		021084	Achriesgill	7	Oct 22	
		021127	Lovstakken	7	Oct 22	
		021138	Gudmedellan	7		
		021140	Veskre	7	Sept 20	
		023416	W. Jemma's Lad	5	Oct 22	Different mtDNA haplotype - possible incorrect pedigree?
012002	Midget	002010	Inca Dove	6	Mar 21	
		021149	Strontian	6	Oct 22	
		049011	Hazel	6	Mar 21	
		049022	Lady Smock	6	Mar 21	
		049025	Nutcracker	6		
		049031	Quercus	6		
		049052	Zostera	6	Mar 21	
		049113	Orchid	6		
		049127	CW. Russet	6	Mar 21	
		049088	CW.Oleander	2	Oct 22	Different mtDNA haplotypes - possible incorrect pedigree?
		049147	CW. Zena	2	Mar 21	
012011	Ladybird	036023	Apple's Original	1		
		320005	A. Roxy	1		
		479021	MG. Jade	1	Mar 21	
		479023	MG. Ruby Rose	1		
		049057	Aspen	6		Different mtDNA haplotypes – possible incorrect pedigree?
		479013	MG. Flint	6	Mar 21	
023001	Old Cricky	023283	Un-named	5		
		023434	W. Brightworthy	5		
023008	Tiny	023279	Tigermoth	4		
		512001	M.Honeybee	4		
023010		109018	P. Ilex	4		
		023203	Aprils Gold	4	Mar 21	
027001	Twilight	000584	A. Gretel	1		
032002	Flicker					Outside UK only
044002	Mountain Lass	044029	Derby Time Lass	61	Oct 22	<6 individuals extant Not breeding Unusual haplotype
048003	Sally					Outside UK only
048029	Willows	021079	Loch na h'Uidhe	7	Oct 22	
		021124	Mallaig	7		
		021142	Flam	7	Oct 22	

054007	Pixie					Outside UK only
076001	Old Altai	002012 049097 049121 049124 049138 049145 049149	Utters Hill Merlyn CW. Quince CW. Rocket CW. Una CW. Yew CW. Zoom	2 2 2 2 2 2 2	Mar 21 Mar 21 Mar 21 Mar 21 Mar 21 Mar 21	
076003	Minnie	900694	A. Neon	9	Oct 22	Founder mare ‘taken on inspection’.

DNA Extraction and Sequencing

DNA extractions and PCR amplification was carried out by a technican (Elizabeth Kilbride) and an undergraduate honours student (Fatema Al Ghaiti), using the detailed protocols described in Appendix 8. In brief, Qiagen DNeasy kits, (DNeasy blood and tissue kit, QIAGEN, Valencia, CA.) following the protocol supplied, were used to extract DNA from the hair samples. 1 462 bp fragment of the D-loop region of mtDNA (Appendix 7, Table A7.1) was amplified using the primers IRD700 5′-CTA GCT CCA CCA TCA ACA CC-3′ and IRD800 5′-ATG GCC CTG AAG AAA GAA CC-3′ (Hill et al. 2002). The PCR was performed with a 20 ul master mix:15.4 ul DNase free water, 2 ul 10 x PCR buffer, 0.6 ul $MgCl_2$ (50mM), 0.4ul dNTP (10mM), 0.2ul IRD700 primer (10mM), 0.2ul IRD 800 primer (10mM), 0.2ul Invitrogen Taq polymerase (5 Units/ul) and 1ul DNA. Thermal cycling was carried out using the following: 5 min at 94˚C; 30 x 60 sec at 94˚C, 60 sec at 50˚C, 60 sec at 72˚C; 5 min at 72˚C. Amplified products were visualised using 2% agarose gel electrophoresis. Sanger sequencing was carried out on an ABI3730 at the University of Dundee sequencing service.

Genomic DNA samples from a subset of 55 the individuals, chosen to include samples from all the available founder matrilines and patrilines, were sent for sequencing to Novogene Inc. UK in three batches between 2020- 2022 and were run on a Novaseq 6000 machine using 150bp paired end sequencing, allowing sequencing of both ends of a fragment to generate alignable sequence data, aiming for 27Gb of raw data to produce 10-20x coverage for each sample. DnaSP v.6.12.03 (Librado and Rozas 2009) was used to collapse the sequences into unique haplotypes.

mtDNA Sequence Data:

Cleaning and Aligning mtDNA Sequences

Cleaning and aligning of the mtDNA sequences was performed by former undergraduate (Fatema Al Ghaiti) and master's (Anna Kinghorn) students. The mtDNA sequences were aligned and manually corrected using *Sequencher* 5.4.6 (Gene Codes Inc, Ann Arbor, Michigan). Forward and reverse sequences were used to create a consensus sequence for each sample. To avoid regions with ambiguities, the 426bp sequences were pruned to 350-bp.

I analysed the data, creating haplotype networks to show the frequency of the mtDNA haplotypes by maternal lineage and by representation in current herds sampled. Two NEXUS files, one arranged by herd of origin, one by matrilinear origin, including a spreadsheet of the newly sequenced Exmoor haplotypes was created, including the herd of origin, and the maternal founder ID as recorded in the EPS Stud Book was used to create a minimum spanning haplotype network utilising PopART (Leigh and Bryant 2015).

Whole Genome Sequence Data:

Processing and analysis of the WGS data was performed by a postdoctoral research fellow (Anubhab Khan) and an MSci student (Alex Brumwell). I visualised and interpreted the results.

Mitogenome Analysis (Alex Brumwell)

Prior to data processing, read quality was assessed using FastQC v. 0.11.8 (Andrews 2010). Trim Galore (Krueger 2021) was used (with default settings) to remove the sequence adaptors and trims the ends of reads. Trimmed reads were mapped to the horse mitogenome reference (NCBI reference: NC_001640.1) using BWA MEM v. 0.7.15 (Li and Durbin 2009). SAMTOOLS v. 1.9 (Li et al. 2009) converted the SAM files into BAM and removed any reads less than average base quality 30. SAMTOOLS *markdup* removed duplicate reads. SAMTOOLS *merge* merged files containing reads from the same individual. PICARD v. 2.8.2 (Broad Institute) using the ValidateSamFile option assessed the validity of the BAMs. QUALIMAP *bamqc* (García-Alcalde et al. 2012) calculated the average coverage of each individual. ANGSD v. 0.916 (Korneliussen, Albrechtsen, and Nielsen 2014) was used with the -doFasta option to call the consensus FASTA for each individual, using the average coverage of each genome as the minimum depth.

A median joining haplotype network (with epsilons set at 0) was created to explore haplotype relationships of complete mitogenomes using PopART (Leigh and Bryant 2015). The network created grouped sequences by their founder matrilines. A quality filter of 500x of coverage excluded two individuals (s49022 and E_21084) from the analysis to obtain higher resolution of the maternal haplotypes. Founder matrilines and herds of origin as recorded in the EPS Stud Book were mapped onto the haplotype network to show the degree of relatedness between the founder lineages (Figure 4.4).

Y- chromosome Analysis (Alex Brumwell)

Fourteen sequences from male animals were used for this analysis. Trim Galore was used to trim the adaptors of the sequences, then the data was mapped using BWA MEM to the most complete horse Y-chromosome assembly up to date (NCBI reference: MH341179.1). SAMTOOLS was used to convert the SAM files into BAM and sorting removed reads below average base quality score 30. SAMTOOLS *markdup* removed duplicate reads. SAMTOOLs *merge* merged files that contained reads from the same individual. PICARD *ValidateSamFile* was used to assess the validity of the BAMs. QUALIMAP bamqc was used to calculate average coverage for each individual sample.

A principal components analysis (PCA) graph investigating the Exmoor pony Y-chromosome was created using the R package *adegenet* (Jombart 2008) and visualised using *ggplot2* (Wickham and Wickham 2016).

Whole Genome Sequence Analyses (Anubhab Khan)

After quality control and trimming as described for the mitogenomes, reads were aligned to the EquCab3.0 equine reference genome (NCBI reference: GCA_002863925.1) using BWA mem. The alignments were then saved in a binary format (BAM) using SAMTOOLS1.9. Duplicate reads were removed with the *samtools rmdup*. Variants were called from the BAM files using Strelka with default options. The variants were filtered with VCFtools to retain biallelic sites with a minimum minor allele count of 3 and remove indels and loci with mean depth across individuals below the 2.5th percentile and above the 97.5th percentile across all loci. All sites with missingness >35% after removing genotypes with genotype quality less than 30 were removed. The X chromosome was removed from the analysis. A total of 299,053 single-nucleotide polymorphisms (SNPs) with mean read depth = 13.05 remained for analyses after these filtering steps.

Estimating Pairwise relatedness

In order to calculate pairwise relatedness based on autosomal (i.e. biparental) data, I used the *--genome* function of *PLINK* (Purcell et al. 2007) to obtain PI_hat estimates based on the SNP data (Guo et al. 2014).

Estimating Inbreeding Based on Runs of homozygosity (ROH) (Anubhab Khan)

To estimate ROH, the filtered SNPs from the autosomal scaffolds were used. Individuals with more than 10× average coverage were grouped by subpopulation (herds). BCFtools/ROH (Narasimhan et al. 2016) was then used to estimate ROH. The autozygous runs obtained were classified into various lengths (runs above 100 kb, 1 Mb, and 10 Mb). The proportion of the genome in ROH (F_{ROH}) for each length was estimated as the total length of the genome in more than the selected length runs divided by the total length of autosomal scaffolds.

The proportion of the genome in ROH above a particular size class was then used to estimate inbreeding arising from ancestors in different historical time periods. The coalescent times of ROH were estimated as $g = 100/(2rL)$, where g is the expected time (in generations) back to the parental common ancestor where the IBD haplotypes form an ROH coalesce. Assuming a recombination rate of 1Mb/centimorgan, the average recombination rate in mammals,100Kb = 500 generations, 1Mb = 50 generations and 10Mb =5 generations, allowing comparison between different generations (Ferenčaković et al. 2013).

Identifying Deleterious Alleles (Anubhab Khan)

In order to compare accumulation of genetic load in Exmoors to other horse breeds, short-read data from whole genome sequences from all the available breeds were downloaded from the sequence read archive on NCBI. Up to five most unrelated individuals for each breed in the dataset, selected by differing pedigree records, were selected. The assumptions described in Khan (2023) were used to identify the deleterious alleles for the modern horses. It is assumed that the population is at fitness maximum, so that missense mutations that change the ancestral amino acid to a very different one are deleterious. Loss of function mutations, i.e., all stop gain nonsense mutations, splice-acceptor or splice-donor variants, are classified as deleterious (Bosse et al. 2019). The sequencing reads were trimmed using *TrimGalore* at default settings. The reads were mapped to the Donkey reference genome assembly (NCBI accession) using *BWA-mem*.

The mapping files were sorted, and the duplicates removed using *samtools*, as described previously. The SNPs were identified using default settings of *Strelka*. The SNPs were filtered as described in Khan et al. (2021) to retain rare SNPs. Any reference allele (i.e. donkey) that was fixed in the Prezwalski horses were classified as ancestral alleles. Any sites not fixed in Prezwalski horses were removed from the analysis. Finally, all reference alleles in the horses were classified as ancestral and all non-reference alleles were identified as derived alleles. The resulting VCF file was annotated using *VEP*. All derived missense and loss-of-function mutations (Xue et al. 2015) were classified as deleterious.

Quantifying Deleterious Alleles Load (Anubhab Khan)

The number of sites with deleterious alleles per individual and the number of sites homozygous for deleterious alleles were counted. These values were normalized with the number of derived synonymous mutations per site. This helped to control for the differences in sequencing depth and missingness. The frequency of mildly deleterious missense alleles (missense/synonymous) and serious loss of function alleles (loss of function count/ synonymous) mutations and the average homozygosity of deleterious alleles (proportion of homozygous loss of function alleles/ synonymous) were then estimated for each individual. These values were compared to levels of recent inbreeding (10Mb FROH) and plotted in relation to mtDNA haplotypes for each individual (as a proxy for matrilines).

Inbreeding and parasite loads

Data showing the parasite loads measured in faecal eggs/g (FEG), averaged from spring and autumn scores recorded over a three-year period (2020 – 2022), was available for twelve of the individuals whose 10Mb F_{ROH} values were known (see Chapter 3). A Pearson correlation coefficient analysis was performed between the average FEG load and the frequency of 10Mb F_{ROH} in individuals, an indicator of inbreeding.

4.8 Results

Pedigree Analysis

In-depth analysis of the studbook records indicated that some of the matrilinear lines have no extant female descendants (i.e., became extinct since the establishment of the post-WW2 population) or that have no direct matrilineal descent, but are represented in the extant population by individuals descended from male descendants of those female founders (Table 2). Although none of these ponies were sampled the table does show that there was further erosion of genetic diversity after the post-war population bottleneck. Five female founder lines became completely extinct since the post-WW2 genetic bottleneck, eight are currently represented only through male line descendants of the female founders (i.e., they are descendants of the male offspring of those female founders), so that the mtDNA lines of those female founders is extinct.

Table 4.2 Founder matrilinear lines with no female descendants extant, i.e. extinct since the establishment of the post-WW2 population, or with no direct matrilineal descent, but with extant individuals represented only through descent from male descendants of those female founders.

Founder ID	Founder name	Notes
001008		Extinct
001016		Extinct
002002	Aconite	No direct female line, but represented by descendants of stallions 002053 Tademus and 009042 F. Beacon
002007	Conkerbelle	No direct female line, but represented by descendants of stallion 002041 Carronade
002030	Carefree	No direct female line, but represented by descendants of stallion 110001 Quartz
012003		No direct female line, but represented by descendants of stallions 012047 Aclander2, 014035 Agrippa and 014043 Trajan.
023004	Kings Crick	No direct female line, but represented by descendants of stallions 023042 Forester, 085020 Bright Boy, 085014 Brackenman
023006		No direct female line, but represented by descendants of stallion 023033 Forest
054005	Bracken	Extinct
071001		No direct female line, but represented by descendants of stallion 002079 Elchi
075001	Just Jennifer	No direct female line, but represented by descendants of stallion 128001 Toadflax
076001	Whortleberry	Extinct
087001	Judy	Extinct

Founder representation

Analysis of the studbook records indicated extensive variation in the number of extant offspring (as of 2022) that can be traced to specific maternal founders (Figure 1). Specifically, 19 founders had >2000 recorded extant descendants, while others had <100. The number of males in this figure refers only to those approved for breeding, thus potential breeding stallions, not castrates or unlicenced males (entire males intended for breeding must be examined at >2 years old and licenced as approved for breeding before their offspring can be registered in the Stud Book).

Of the 41 foundation mares registered in the 1980 EPS Stud Book, only 28 now have direct matrilinear line female descendants extant, with 24 of these represented in the UK population (Table 4.1). Eight of these foundation mares are recorded as still represented in the current population only through the descendants of stallions born into their matrilineal lines and five of these founder mares have no registered living descendants, their lines being functionally extinct (Table 4.2). Of the 28 recorded matrilinear lines with female descendants still represented on the current Stud Book, 27 are from foundation mares whose ancestry was from herds registered in pre-1980 stud books and one is descended from a mare registered by the ‘taken on inspection’ route.

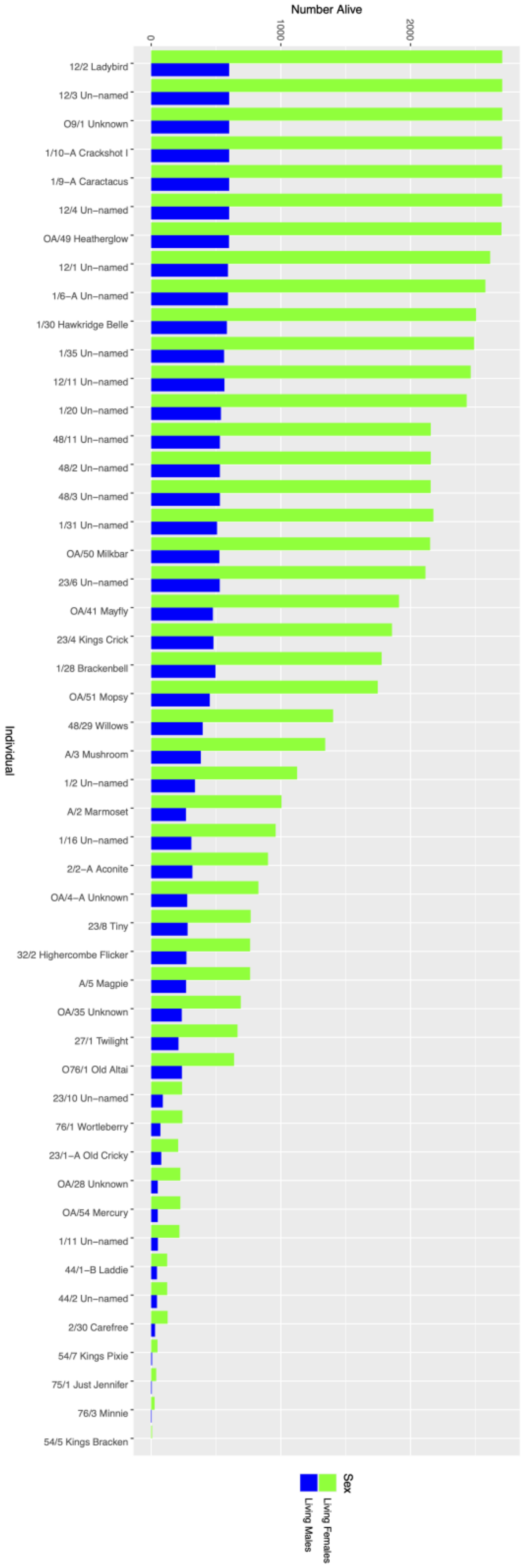


Figure 4.1 Uneven representation of the founder lines in the extant population (as of 2022). X-axis shows the individual founder lines, y-axis shows the numbers of live females or potentially breeding stallions whose pedigrees can be traced to specific founders.

Effective breeding population

The effective breeding population (*Ne*) values were calculated from Exmoor Pony Stud Book records from 1972- 2022 and were compared with the total foal registrations from the same period. Foal registrations and the effective breeding population rose from the late 1970s until peaking around 2010. A subsequent negative trend has led to the 2022 registrations and effective population size being reduced to a level similar to the early 1980s (Figure 4.2).

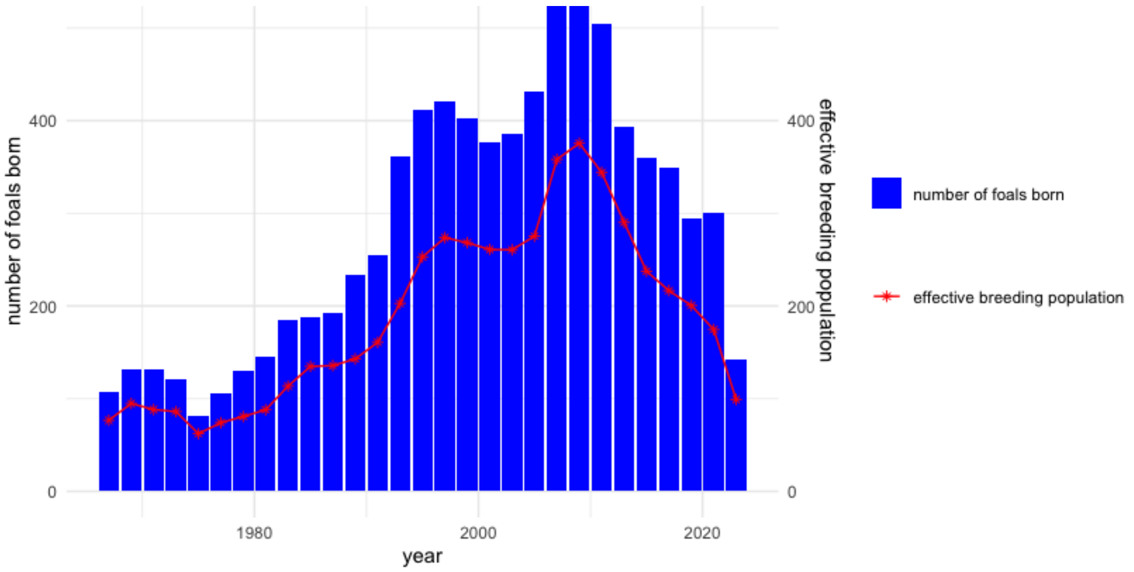


Figure 4.2 Effective breeding population (Ne) values calculated from Exmoor pony Stud Book records from 1972- 2022 (red line), compared to the number of foals born (blue histogram). X-axis shows the year, the y-axes show the number of foals born on the left and the Ne on the right.

The variation in offspring remaining across founder lines also meant that there was extensive variation in the number of individuals that I was able to sample from each matrilineage (Figure 3). Herd 900, the Anchor herd, has the greatest female founder diversity, with eight founder matrilines sampled. Herds 49 and 21 showed the highest frequencies of individuals from specific founder matrilines, from 12002 and 1020 respectively.

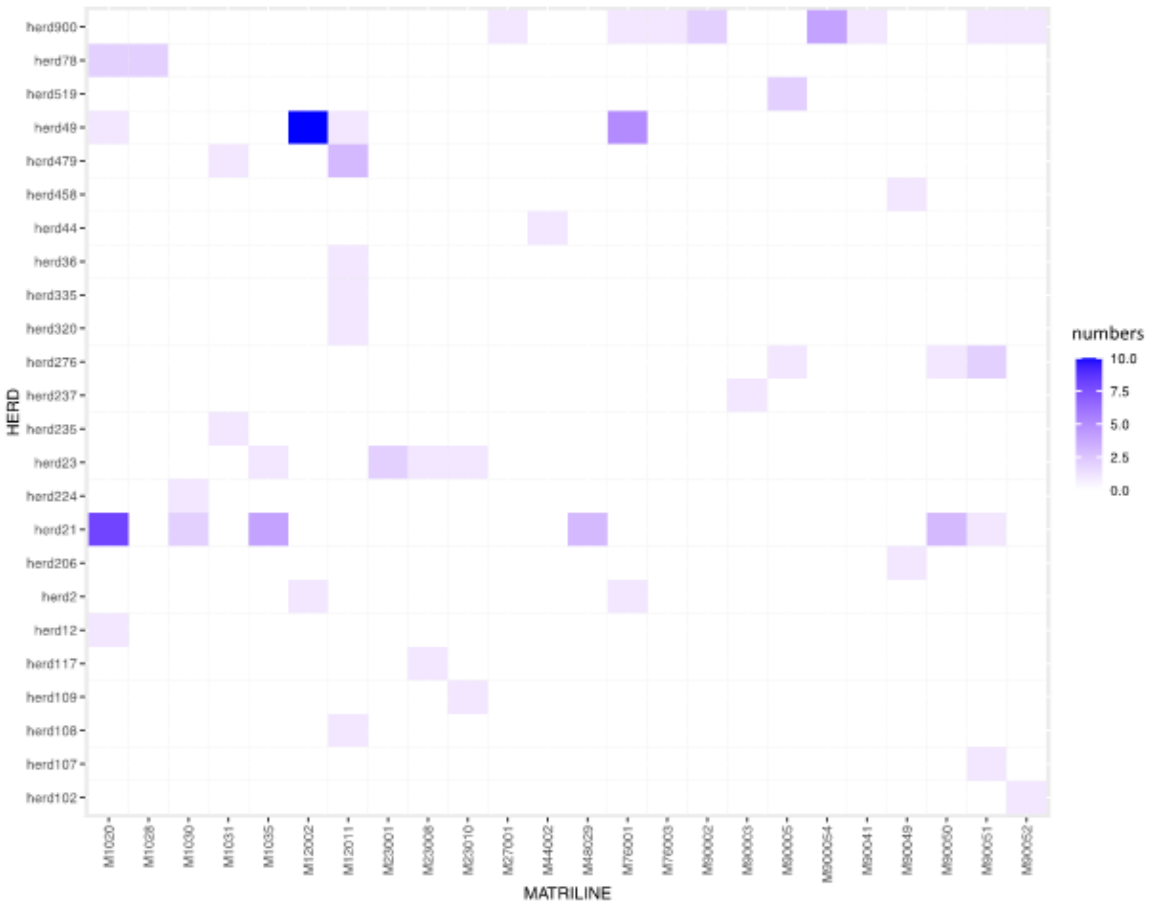


Figure 4.3: Heatmap showing the frequency distribution of individual ponies descended from specific founder matrilines by herd within the sample set. x-axis shows the herds, y-axis shows the matrilines. The intensity of colour indicates the number of individuals sampled.

mtDNA Analyses

A total of seven D-loop mtDNA haplotypes were resolved from 80 individuals sampled from across herds and maternal lineages (Table 4.1). Three female founder lines (1035, 12002 and 12011) apparently had descendants with multiple mtDNA haplotypes (1035 = haplotypes 5 and 7, 12002 = haplotypes 2 and 6, 12011 = haplotypes 1 and 6) (Figure 4.4). These findings are inconsistent with the sampled ponies from each recorded matriline actually sharing descent from the same foundation matriline, (Table 4.1), as it is not possible for ponies descended from the same matrilineal line to have different mtDNA haplotypes.

A median joining haplotype network organised by maternal founder lineages demonstrated extensive sharing of mtDNA haplotypes across maternal lines but uneven representation of haplotypes in the extant population (Figure 4.4). Since different herds tend to be dominated by different individual maternal lines, the pattern was similar for a median joining haplotype network organised by herds, with Herd 49 showing the greatest haplotype diversity (with ponies carrying haplotypes 7, 5, 6 and 2) (Figure 4.5). Haplotype 7 was most frequent in the sampled population and dominates Herd 1 and Anchor herd (900) matrilines (1020, 1030, 1031, 1035, 48029, 900002, 900003, 900005, 900041, 900049, 900050, 900051, 900052, 900054), which are currently represented in 15 herds but most frequently in herd 21 (Figure 4.5). Haplotype 9, represented by a single pony from maternal lineage 76003 (Figure 4.4), is separated from haplotypes 7 and 5 by ten or nine mutations respectively, with haplotypes 7 and 5 being themselves separated by 6 mutations. Haplotype 5 is found in Herd 1 and Herd 23 (Figure 4.4) matrilines and is separated from haplotypes 1 (found in two matrilines maintained in six different herds) and haplotype 4 (found in two matrilines maintained in three herds) by 6 mutations. Haplotype 1 is found in the 012011 and 27001_matrilines Haplotype 1 is joined to haplotype 2 by a branch with 2 mutations and haplotype 6 by a branch with 10 mutations.

The haplotype networks also visualise the anomalies described in Table 4.1; each maternal line is expected to be represented by only a single mtDNA haplotype but in some instances they are represented by more than one highly distinct haplotypes. Haplotype 2 is found most frequently in the 76001 matriline but was also in two individuals from the 12002 matriline, most of which have haplotype 6 (separated from haplotype 2 by 11 mutations). Haplotype 6 was also found in two ponies recorded as from the 120011 matriline, although haplotype 1 (separated from haplotype 6 by 9 mutations)_was found in most individuals from that matriline. A single individual from matriline 1035 had haplotype 5, even though most individuals had haplotype 7 (separated by 6 mutations).

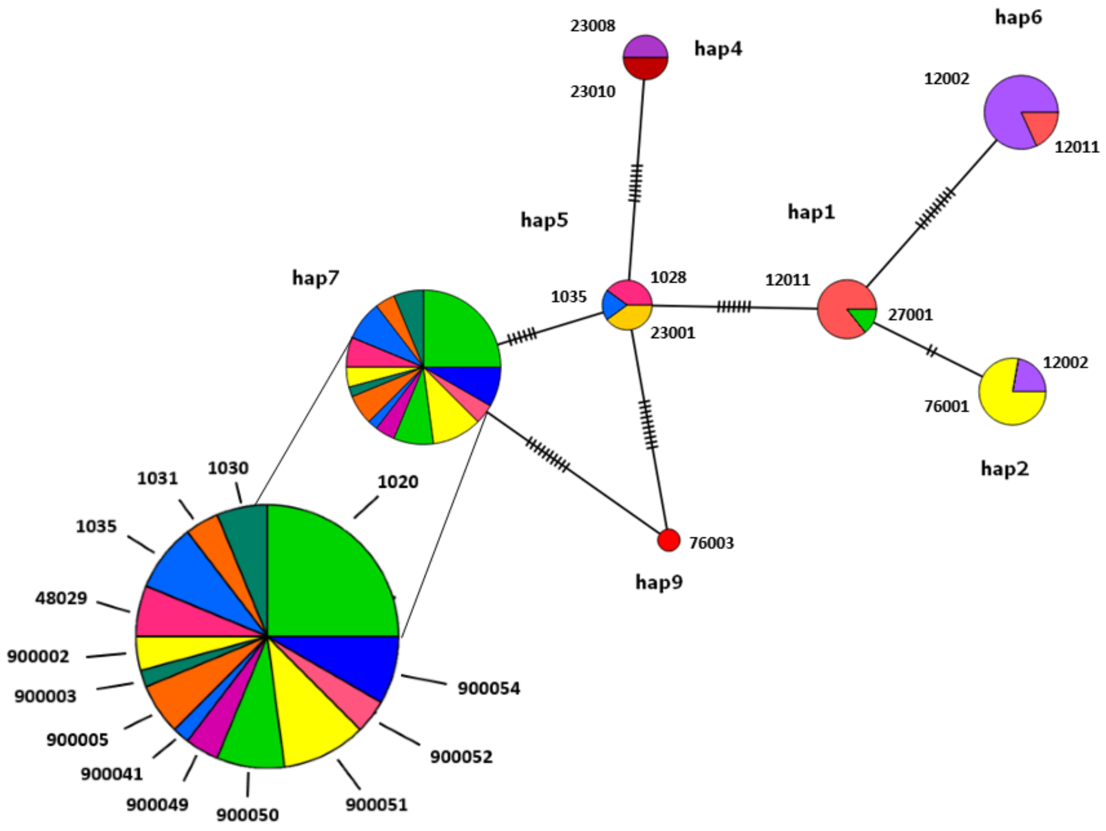


Figure 4.4 mtDNA haplotype network displayed by founder matrilines: Circle size is proportional to the relative frequency of haplotypes. Matrilineal lines are shown in differing colours. The number of mutations separating haplotypes is indicated by cross-hatching along the network branches, indicating the number of mutations separating haplotypes. Each haplotype is labelled to show the maternal lines where it occurs. Haplotype 7, the most frequently occurring haplotype, is expanded to allow labelling of the 14 maternal lines where it occurs.

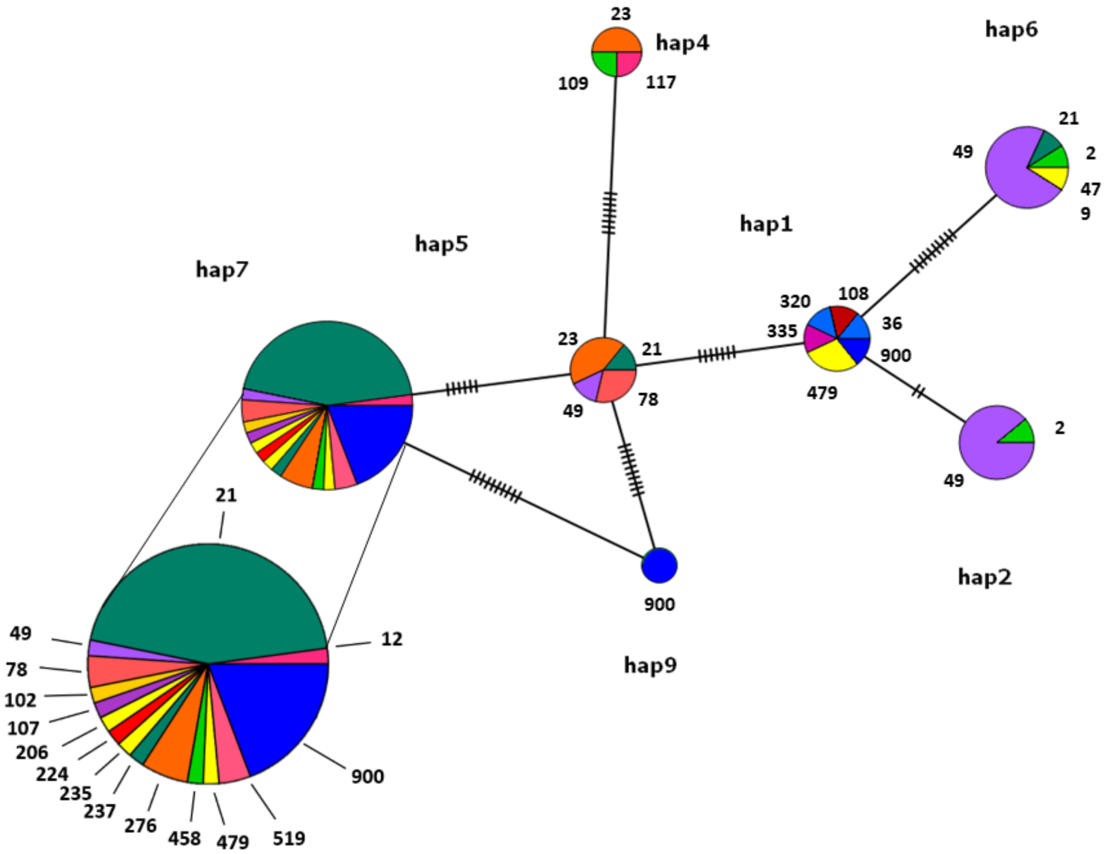


Figure 4.5 mtDNA haplotype network displayed by current, sampled herds: Circle size is proportional to the relative frequency of haplotypes. Herds are shown in differing colours. The number of mutations separating haplotypes is indicated by cross-hatching along the network branches indicating the relative genetic distance between haplotypes. Each haplotype is labelled to show the herds where it where the sampled ponies originate. Haplotype 7, the most frequently occurring haplotype, is expanded to allow labelling of the 15 herds where it occurs.

Y-chromosome Analysis

An investigation of y chromosome variation in the population using samples from 13 males representing descent from a wide range of the male founder pedigrees. Most of the individuals clustered in a group, but two individuals were separated, sample E107013 being separated along PC1 and, sample E21084 being separated along PC2 (Figure 4.6). However, when compared with the published y chromosomes from Prezwalski horses and domestic horse breeds, most Exmoors clustered within a group of domestic horse breeds, along PC2, with one sample as an outlier to the other Exmoor samples on PC1. All the Exmoor samples were within the range of the domestic horse breeds. Two of the Prezwalski samples were separated from the rest of the samples on PC2 (Figure 4.7).

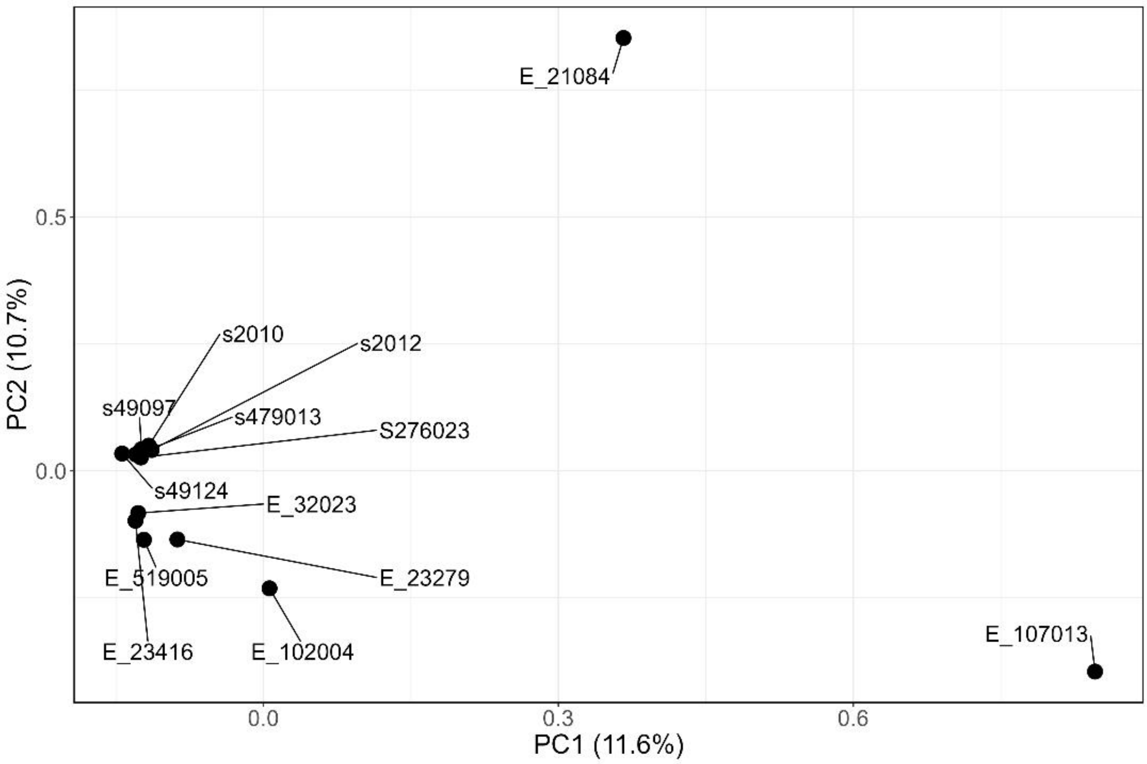


Figure 4.6 Y-chromosome variation within Exmoors. Most samples cluster together but one sample is separated on PC1, and one individual is separated on PC2.

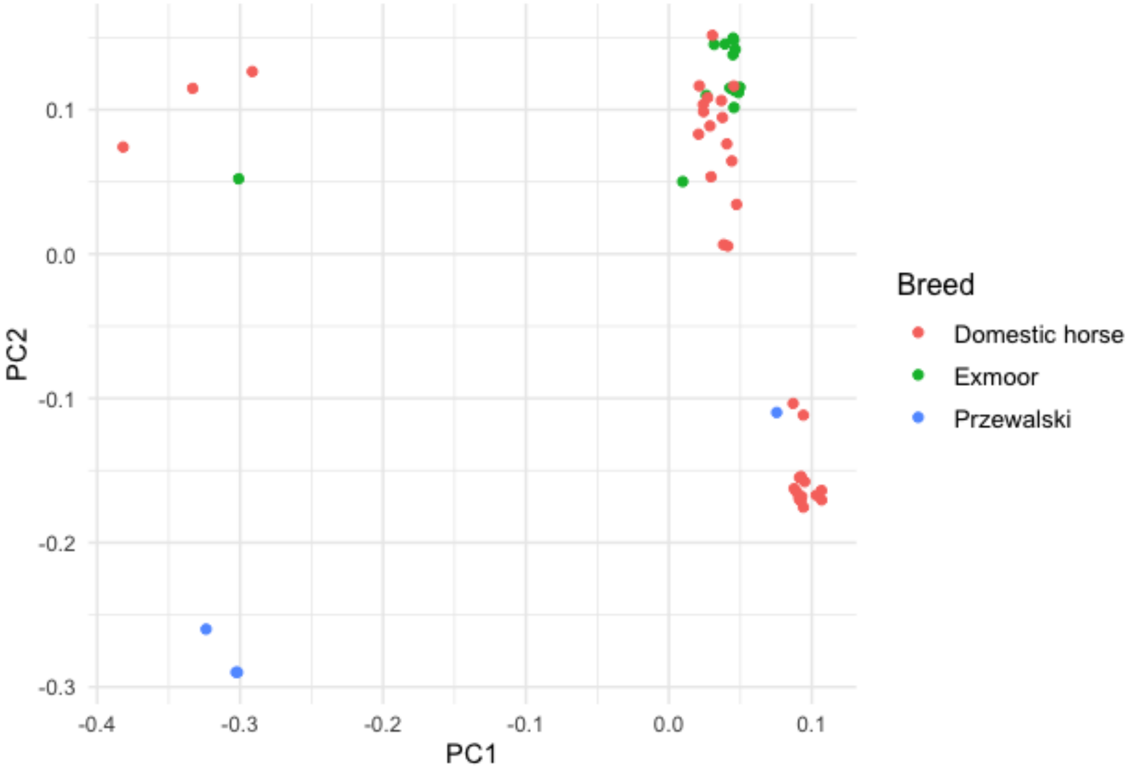


Figure 4.7 Y-chromosome variation between Exmoors, Prezwalskis and domestic horses. Exmoor samples are shown in green, Prezwalski samples in blue and domestic horse breeds in red. Most Exmoor samples cluster together, one is separated on PC1. All Exmoor samples were within the range of the domestic horse breeds.

Bi-parental Pairwise Relatedness

Average relatedness of individuals within herds, as determined by Pi-Hat, based on the sampled herds where >3 ponies were sampled is shown in Figure 4.8. The boxplot shows the variance of pairwise relatedness values around the median and individual outliers by herd. Herd 49 had the highest range of outliers. A Mann Whitney U test with a Bonferroni Correction to adjust the p-value threshold for significance based on the number of comparisons being made was applied to investigate significant differences in Pi-Hat values between herds. Herd 23 was shown to be significantly different, based on pairwise relatedness, from the other sampled herds (Figure 4.9). No other herds were significantly different from each other.

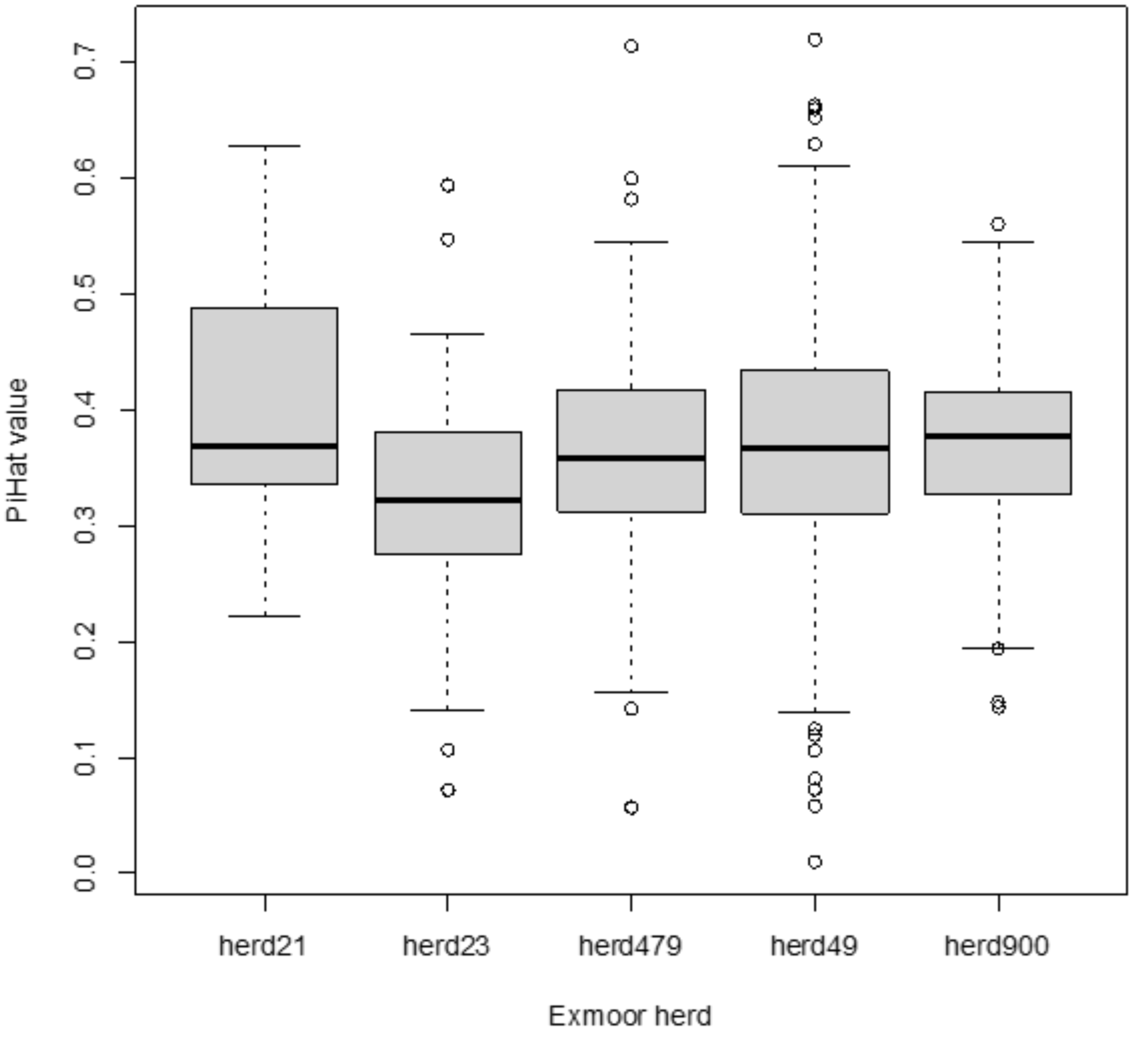


Figure 4.8 Boxplot showing pairwise relatedness (Pi-Hat values) from herds with >3 sampled ponies.

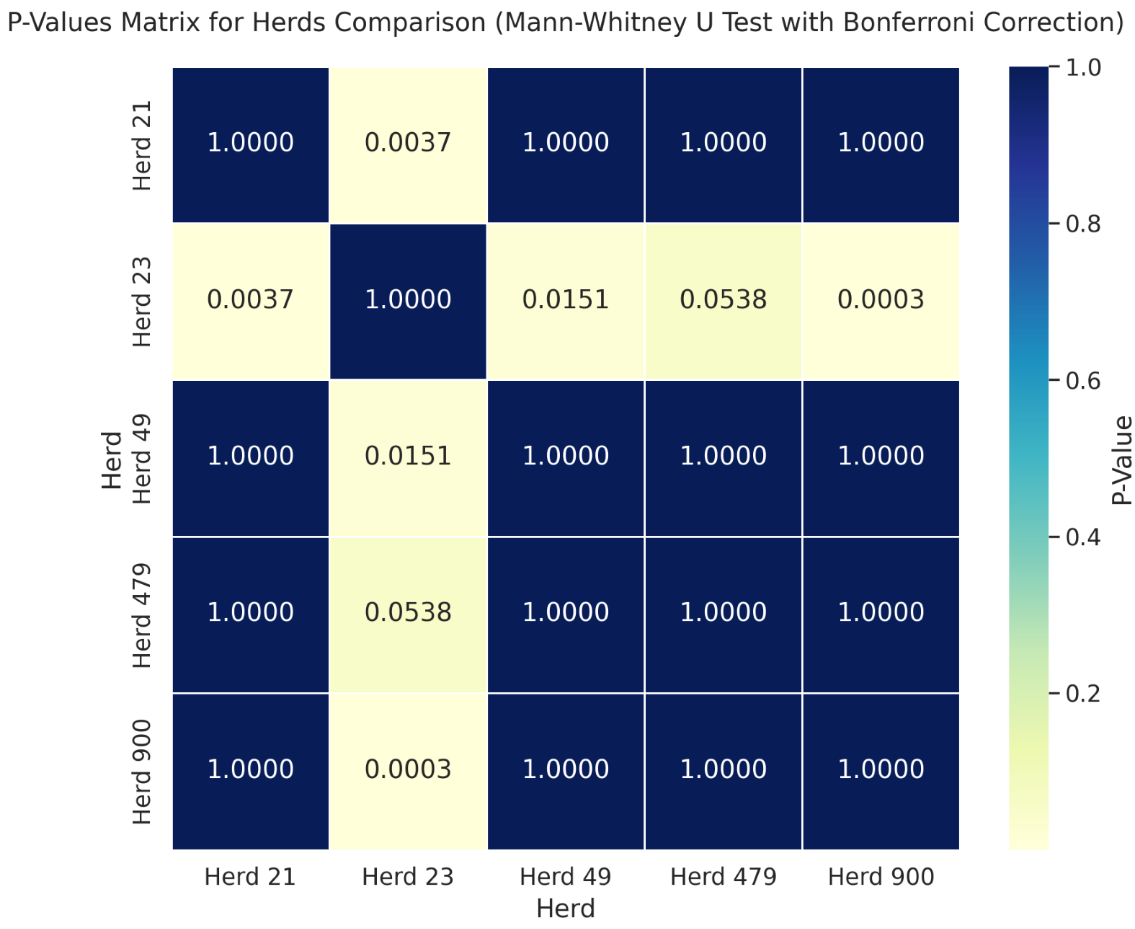


Figure 4.9 P-value matrix from herds with >3 sampled ponies showing significant differences in mean Pi-hat values between herds. Herd 23 is shown to be significantly different, based on pairwise relatedness, from the other herds.

Inbreeding Based on ROH

The results of a Tukey's test for significant difference on the 100Kb F_{ROH} values (i.e. historical inbreeding, estimated as 500 generations) between the Exmoor ponies sampled with other 23 other domestic horse breeds (Akhal Teke, Arabian, Dutch Warmblood, Franches Montagnes, German riding pony, German Warmblood, Haflinger, Hanoverian, Holstein, Icelandic, Morgan, Noriker, Oldenberg, Paint, Quarter Horse, Shetland, Standardbred, Swiss Warmblood, Thoroughbred, Trakehner, UK Warmblood, Welsh pony and Westfale) breeds published on the National Centre for Biotechnology Information (NCBI) data base (Figure 4.10) shows that_Exmoors have significantly higher inbreeding than three of the other breeds (Franches Montagne, p=1.048495e-12, German Riding pony, p= 2.277524e-03, and German Warmblood, p=9.162305e-03).

The results of a Tukey's test for significant difference showed the greater distinctiveness of Exmoors increased for more recent inbreeding, at the 1Mb F_{ROH} values (i.e. historical inbreeding, estimated as 50 generations) (Figure 4.11) where only the Haflinger shows overlapping ranges. Exmoors at the 1Mb F_{ROH} values show significantly lower inbreeding than Akhal Tekes and Arabians, but higher than Franches Montagne, Hanoverians, Icelandics, Morgans, Norikers, Oldenbergs, Shetlands, Welsh ponies and Westfales. Haflingers show significantly more inbreeding than the Franches Montagne and German riding pony at the 1Mb F_{ROH} value.

For the most recent inbreeding, 10Mb F_{ROH} values (i.e. historical inbreeding, estimated as 5 generations) (Figure 4.12), Exmoors were still significantly less inbred than the Akhal Tekes and Arabians that they were compared with, but were significantly more inbred than the German riding pony, Hanoverian, Icelandic, Oldenberg, Trakehner and UK Warmblood. Exmoors had no significant difference in recent inbreeding from the other breeds that they were compared with (Dutch Warmblood, German Warmblood, Haflinger, Holsteiner, Morgan, Noriker, Paint, Quarter Horse, Shetland, Standardbred, Swiss warmblood, Thoroughbred, Welsh pony and Westfale).

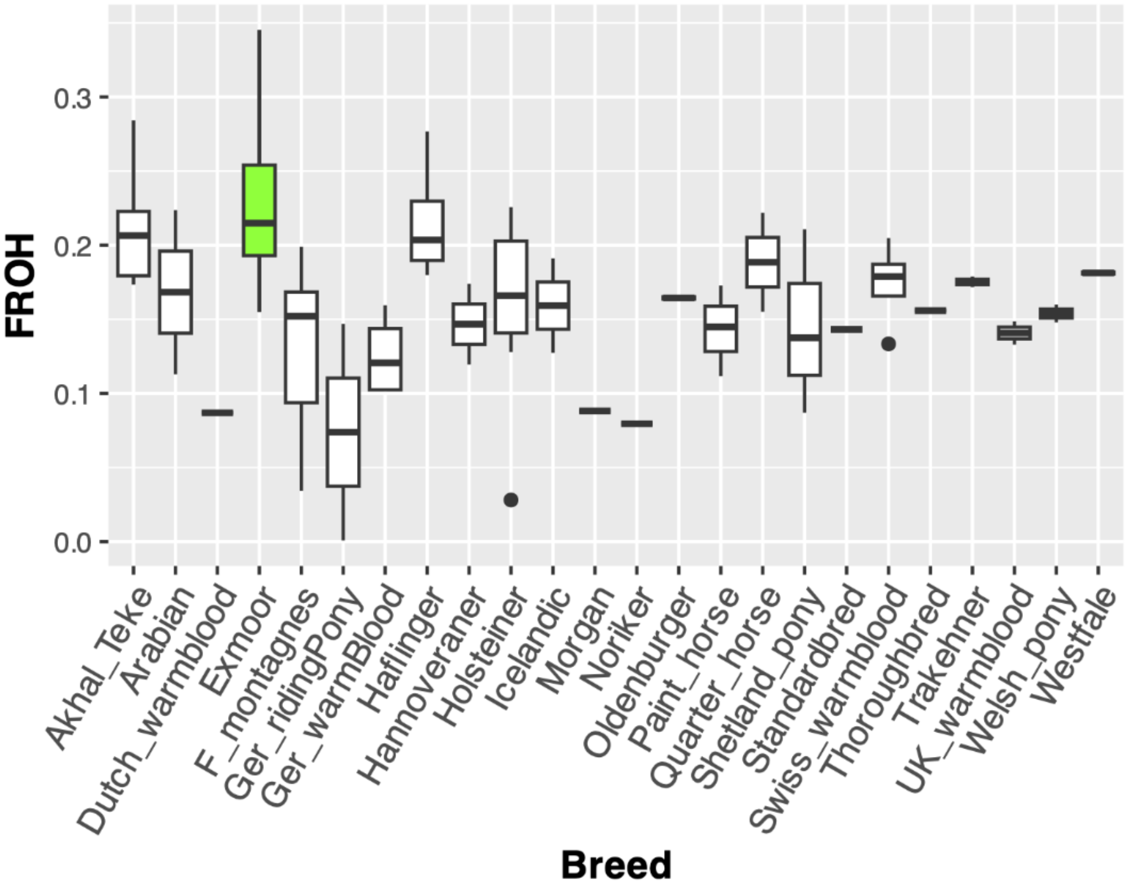


Figure 4.10 Variation in median inbreeding at the 100Kb F_{ROH} values of Exmoor ponies compared to 23 other breeds. A Tukey's test shows Exmoors have significantly higher inbreeding than the Franches Montagne, German riding pony and German Warmblood.

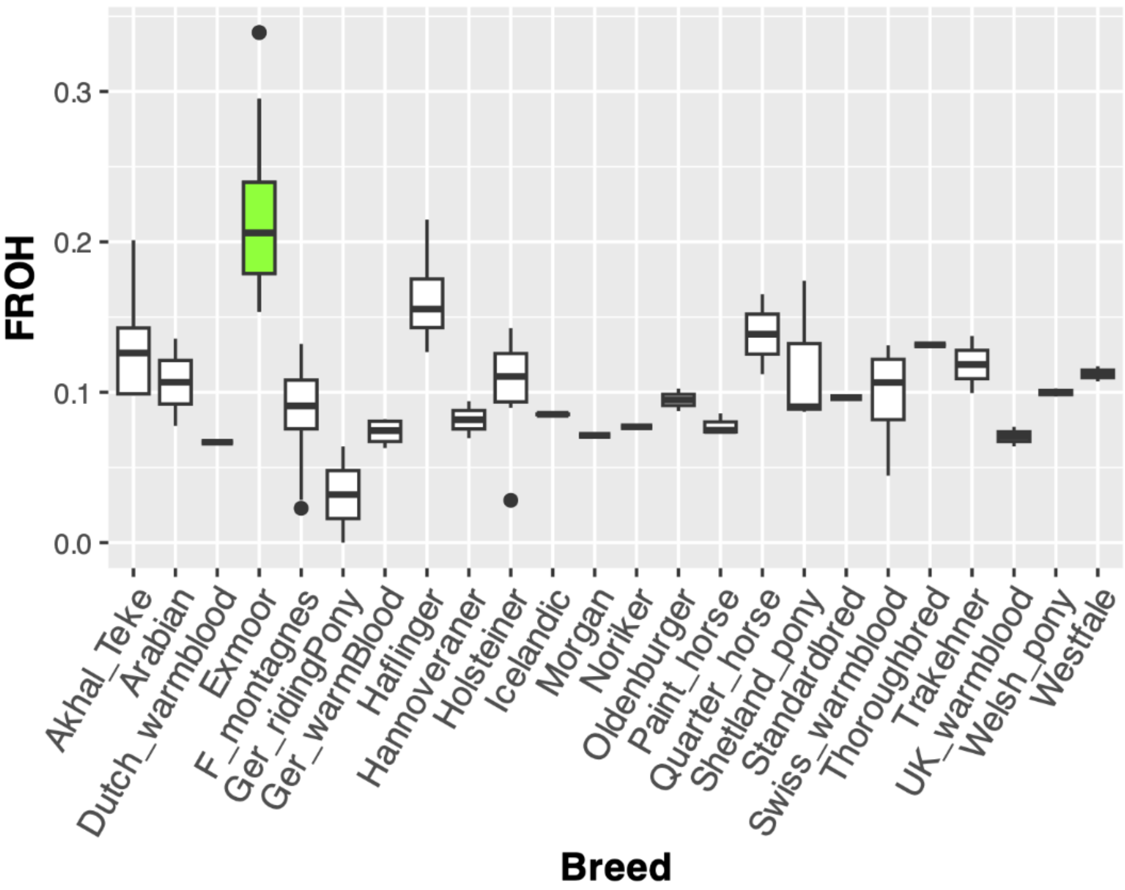


Figure 4.11 1Mb F_{ROH} values of Exmoor ponies compared to 23 other breeds. Exmoors at the 1Mb F_{ROH} values show significantly lower inbreeding than Akhal Tekes and Arabians, but higher than Franches Montagne, Hanoverians, Icelandics, Morgans, Norikers, Oldenbergs, Shetlands, Welsh ponies and Westfales.

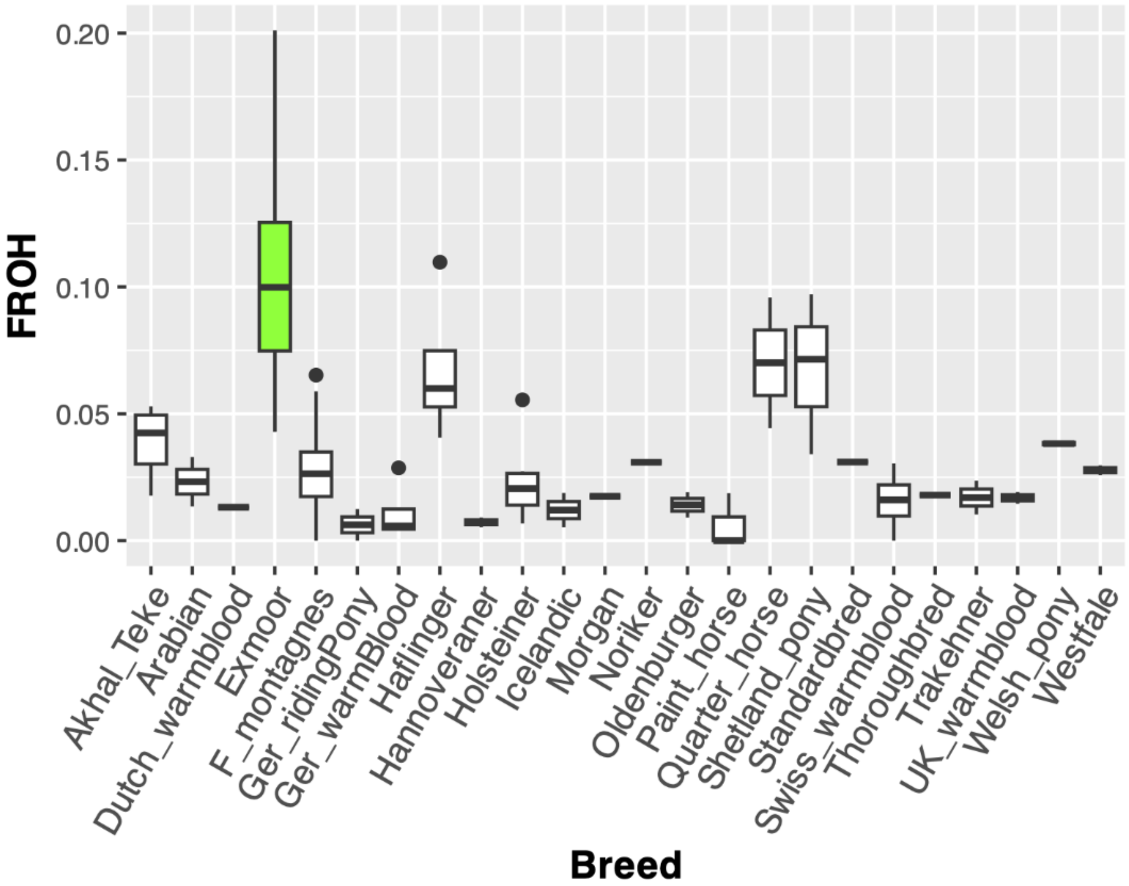


Figure 4.12 10Mb F_{ROH} values of Exmoor ponies compared to 23 other breeds. Exmoors were significantly more inbred than the German riding pony, Hanoverian, Icelandic, Oldenberg, Trakehner and UK Warmblood.

A comparison of 10Mb F_{ROH} frequencies (recent inbreeding) between sampled Exmoor visualised by herd shows that there is considerable variation within and between herds (Figure 4.13). The lowest 10Mb F_{ROH} was found in herd 335 (frequency 0.06), although only one individual from that herd was sampled, so this might not be representative of the herd overall. The highest 10Mb F_{ROH} was found in herd 21 (frequency 0.20), and although the average of the samples from the herd was lower, the range was the greatest (average = 0.10, range 0.03 to 0.20, n=8). An individual from herd 900 had the lowest average frequency (average = 0.07, range 0.02 to 0.11, n= 7). Of the herds where multiple individuals were sampled, herd 23 had the highest average frequency (average = 0.13, range 0.09 to 0.17) but there was extensive variation among individuals.

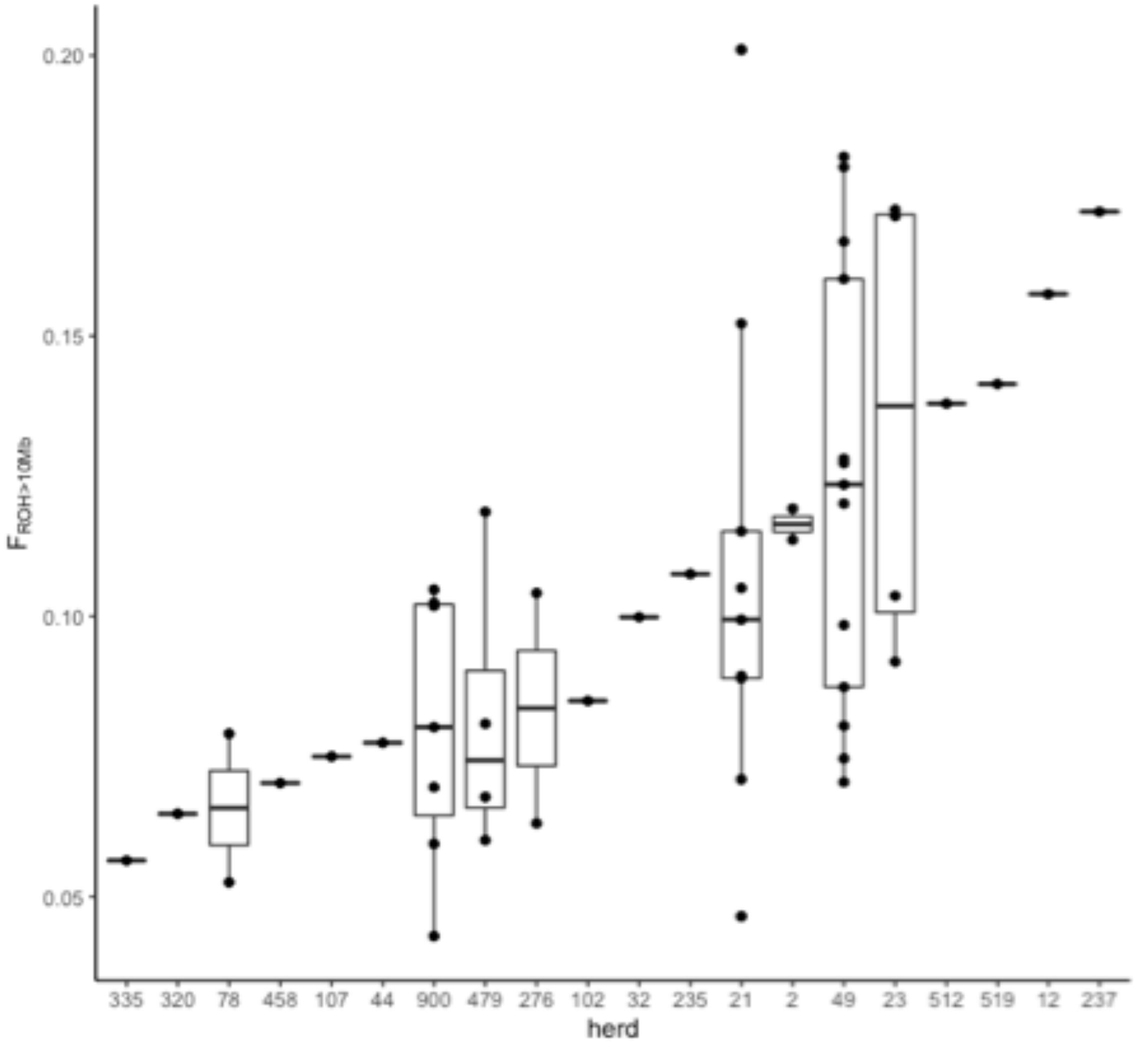


Figure 4.13 10Mb F_{ROH} values of Exmoor ponies compared by herd. X axis shows herds, y-axis shows 10Mb F_{ROH} values.

Genetic Load in Relation to Inbreeding

Based on estimates of loss of function mutations (LOF) standardised by synonymous derived mutations, there was a significant decrease in LOF mutations with increased inbreeding (R=-0.29, p= 0.03) but clustering of mtDNA haplotypes was observed (Figure 14). A similar trend was found for mildly deleterious mutations (missense alleles), although it was not significant (R=-0.18, p= 0.19) (Figure 15). Although also not significant (R=0.18, p= 0.18), there was weakly positive correlation between the proportion of the LOF mutations that were homozygous and inbreeding (Figure 4.16).

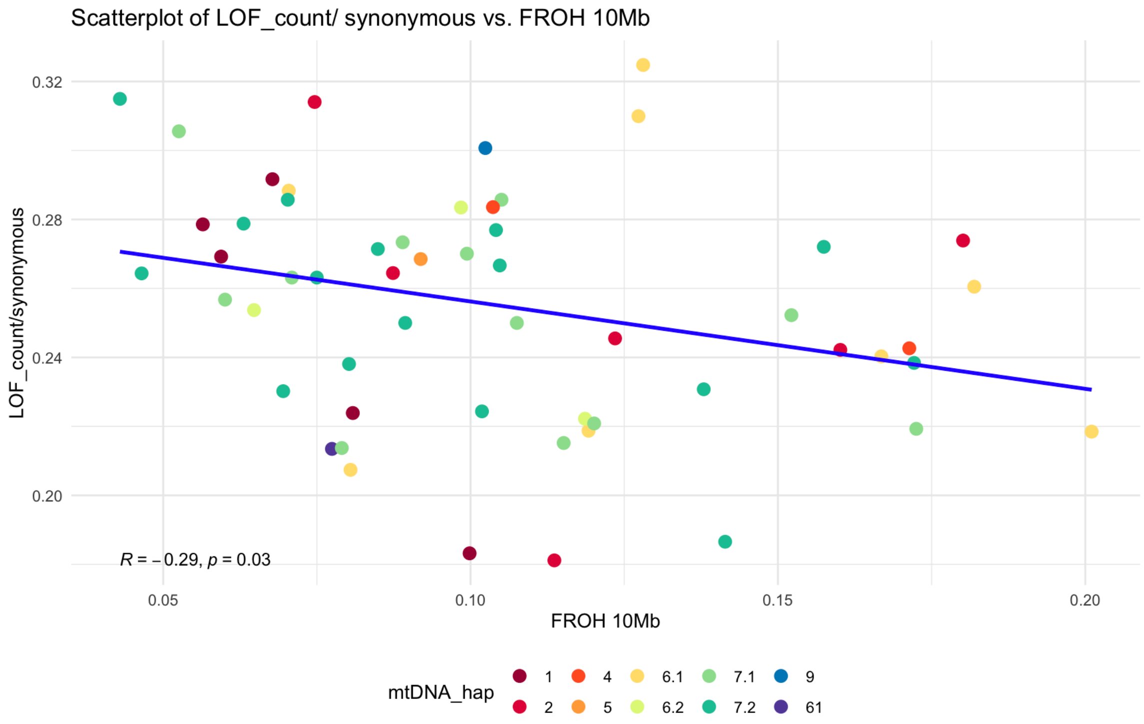


Figure 4.14 Scatterplot of the count of loss of function (LOF) alleles relative to the count of synonymous alleles with increased recent inbreeding, as measured by the percentage of 10Mb FROH. X axis shows inbreeding, Y axis shows the count of loss of function alleles relative to the count of synonymous alleles. Data points are plotted by mtDNA haplotypes, as a proxy for matrilines (see Appendix 9, Table A9.1).

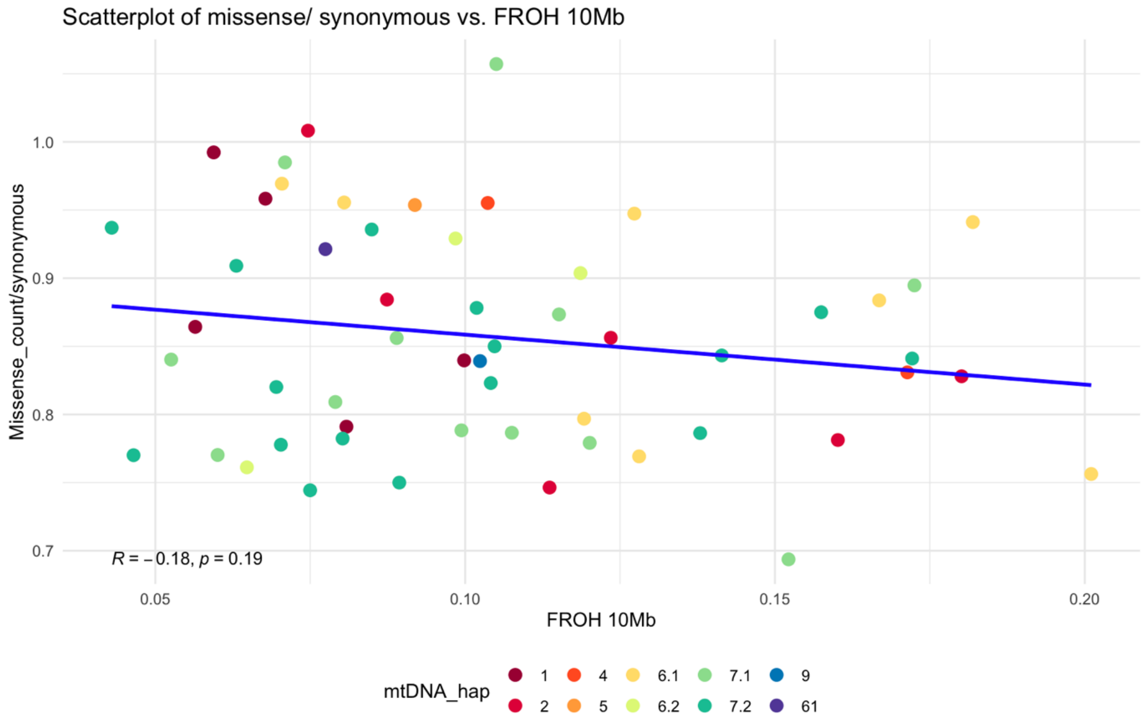


Figure 4.15 Scatterplot of the count of missense alleles (predicted to be mildly deleterious) relative to the count of synonymous alleles with increased inbreeding as measured by the percentage of 10Mb FROH. X axis shows inbreeding, Y axis shows the count of missense alleles relative to the count of synonymous alleles. Data points are plotted by mtDNA haplotypes, as a proxy for matrilines (see Appendix 9, Table A9.1).

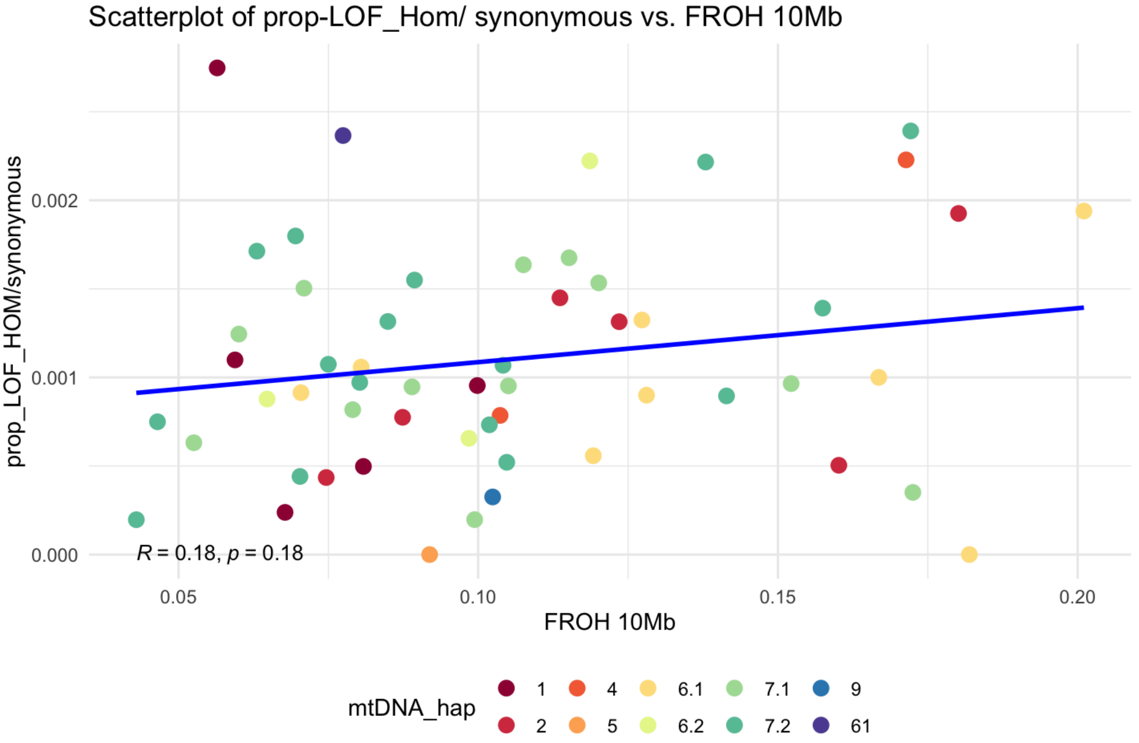


Figure 4.16 Comparison of the proportion of loss of function alleles relative to the proportion of synonymous alleles with increased inbreeding as measured by the percentage of 10Mb FROH. X axis shows inbreeding, Y axis shows the proportion of loss of function alleles relative to the proportion of synonymous alleles. Data points are plotted by mtDNA haplotypes, as a proxy for matrilines (see Appendix 9, Table A9.1).

Parasite Loads in Relation to Inbreeding

Inbreeding was significantly positively correlated with FEG (correlation = 0.803, p= 0.03, n=12; Figure 4.17). The most inbred individual was an outlier, with no detected parasite load (FEG= 0, >0.20 10Mb FROH).

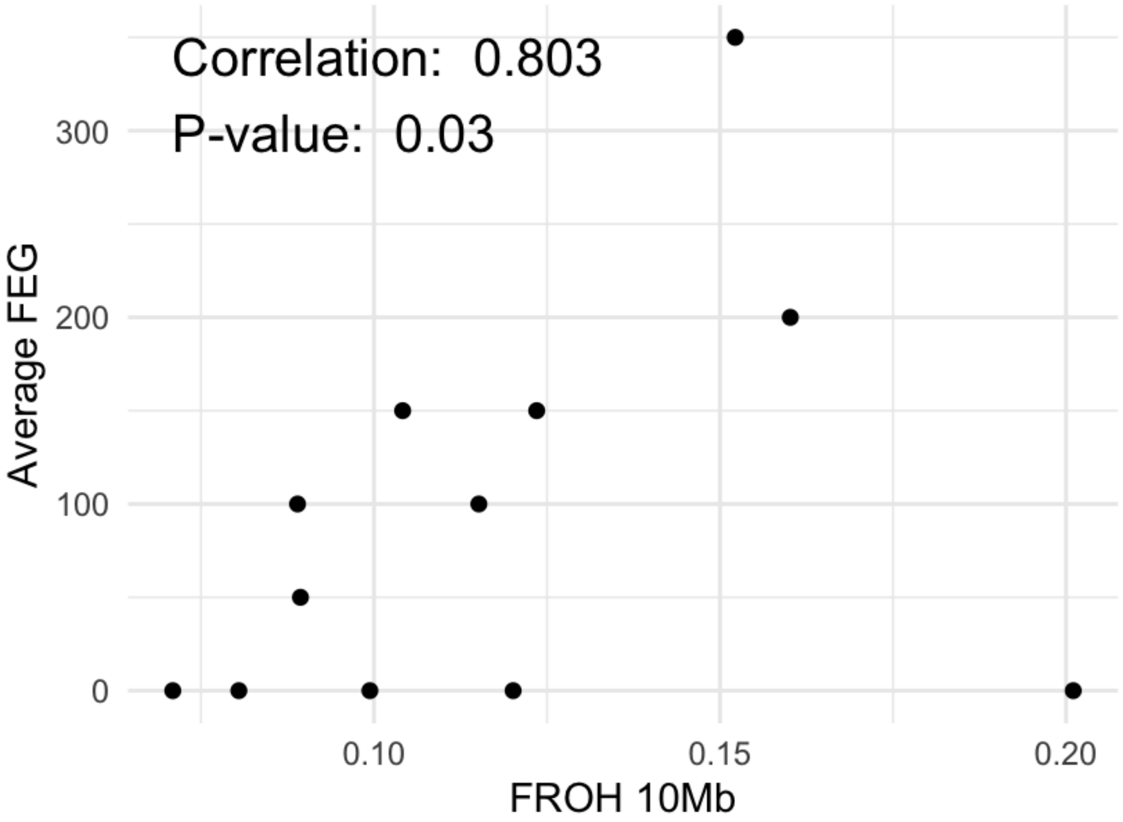


Figure 4.17 The relationship between individual inbreeding and parasite loads. X axis shows recent inbreeding as measured by the percentage of 10Mb FROH. Y axis shows parasite load in faecal eggs per gram (FEG).

4.9 Discussion

Pedigree Analysis

The genetic investigation of matrilines identified using Stud Book data showed considerable congruence, with few anomalies from the expected results amongst the sample set. The samples were selected to offer a cross-section of the entire extant UK Exmoor pony population and can therefore be of interest to all breeders. Only one foundation matriline was not sampled for logistical reasons. The clustering of founder lines by mtDNA haplotype has mostly aligned as expected according to the location and management history of those foundation matrilines, indicating that most recording of maternal pedigrees has been accurate, even for free-living herds, as has been found in similar studies of Soay sheep where observation records of maternal parentage are usually accurate (Hunter et al. 2019; Bérénos et al. 2014).

Analysis of the pedigrees suggested that there has been further erosion of the founder Exmoor pony population since the genetic bottleneck that occurred in WW2. Investigation into the representation of matrilineal and patrilineal founder lines in the potential breeding population showed that there is considerable unevenness of representation between them among extant individuals. Representation of the founder lines in live females is higher than in live males because all mares can potentially reproduce and only breeding individuals are represented. Some founders had few or no breeding male descendants, likely to further decrease the future representation of that founder line in the population since a stallion will probably produce more offspring in his lifetime than any individual mare. It seems probable that, without intervention, some currently extant founder lines may become extinct in the future, whilst others are apparently thriving. Ongoing monitoring of founder representation would allow breeders to act to prevent further loss of founder representation, maintaining genetic diversity within the population.

Monitoring of changes in the effective breeding population would be one indicator of likely changes in inbreeding in the population. Using pedigree data from the online Stud Book data from 1972 – 2022. to the effective breeding population size remained <100 until 1983, when an increase to a peak of >350 in 2009 was observed, followed by a decline to <100 in 2022, the most recent data available (Figure 2). Monitoring of this negative trend is required, with investigation into its probable causes, so that appropriate advice may be offered to breeders to mitigate potential associated declines in genetic diversity within the population.

Mitochondrial DNA analysis

The use of mtDNA haplotype data can be of use inform conservation priorities (Antunes et al. 2007; Mucci et al. 2010; Rosauer et al. 2016; Zink et al. 2010). There has been historic reduction in the mtDNA haplotypes over time: of the 87 mtDNA haplotypes found in ancient equines, only 39 exist in modern breeds, probably due to post-domestication breeding selection (Cieslak et al. 2010).

The mtDNA analysis based on the D-loop indicated that seven mitochondrial haplotypes are present in the sampled UK Exmoor population, although the mitogenome analysis suggested that there could be additional substructuring within these. Studies of European bison, (*Bison bonasus*), found only three distinct mtDNA haplotypes after a recent genetic bottleneck (Wójcik et al. 2009), Arctic foxes, (*Vulpes lagopus*) were also found to have lost four of their seven mtDNA haplotypes post-bottleneck (Nyström, Angerbjörn, and Dalén 2006). Unfortunately, samples from the founder matrilines that became extinct since the Exmoor population bottleneck were not included in this study, so information on the possible recent loss of haplotypes is not available.

The distribution of the existing haplotypes is uneven in the population, with haplotype 7 being most numerous and haplotype 9 being found in a single individual. Although the haplotype frequencies may be influenced by sampling bias, selection was made to include samples from all the recorded matrilineal lines (with only one for which no samples were available), to include a diverse selection within the entire UK Exmoor pony population. The mtDNA data provided a good test for the accuracy of the studbook records. Haplotype 7 is found uniquely in 12 matrilines, descended from Herds 1, 900, (Anchor) and 48, now found in 15 current herds. This is unsurprising as these herds were known to have some shared management historically. The lack of haplotype variation in these matrilines supports recorded evidence of their single founder female origins.

Eleven ponies recorded as descended from 12002 matriline were sampled: nine had haplotype 6, but two, a mother and daughter, had a haplotype separated by 11 mutations (haplotype 2). This could indicate that the founder matriline actually had more than one foundation mare and could be split into multiple matrilines, or that pedigrees may have been incorrectly recorded. On personal consultation with the breeder, the two ponies with haplotype 2 were actually found be descended from the 076/1 founder line, which had haplotype 2. A mistake in the recording of the origin of the mother's sample had occurred due to two ponies registered into the herd having the same name. This lack of haplotype variation in the 12002 matriline supports recorded evidence of its single founder female origin.

Similarly, of the six sampled ponies recorded as descended from matriline 12011, four had haplotype 1 and two had haplotype 6, which was associated with matriline 12002. The two founder mares from herd 12, (12011 and 12002) were kept together, and were believed to be closely related, although this is belied by the haplotype network, which shows them to be separated by nine mutations in the D-loop. Many herds have only one stallion present at any time so, as they were of similar age and bred in the same herd, they are highly likely to have shared paternal ancestry. It_is possible that the offspring were misassigned due to physical similarity between the mothers. The NPS Stud Book 1919 shows Herd 12 as having four foundation mares, although only three, 12002, 12003 and 12011 are recorded in the 1980 EPS Stud Book. Founder mare 12003 was breeding post-WW2, but is recorded as having left only male descendants. If this record is incorrect and there were female descendants from her mistakenly attributed to the 12011 matriline, and she was haplotype 6, that could explain the origin of the two ponies with an anomalous haplotype from the 12011 matriline. Further investigation would be required to clarify the possibilities.

One of the ponies that we sampled, registered into herd 23 that was recorded in the studbook as descended from matriline 1035 had haplotype 5. However, the_four other individuals descended from matriline 1035 had haplotype 7. Other ponies sharing haplotype 5 come from the 1028 and 23001 matrilines (a herd 23 foundation line); which suggests a close relationship between founder herds 1 and 23. This seems unlikely and suggests that perhaps there was an error in recording the pedigree. Separated by 13 mutations from haplotype 7 (the Herd 1 and Anchor herd ponies) and managed discretely, they are often phenotypically different, more usually having bay or light bay coats, rather than the brown or dark brown coats frequently seen in other herds (personal observation and Baker, 2008). The other herd 23 matrilines 23008 and 23010 both had haplotype 4, which in Exmoors was found to be unique to ponies of herd 23 descent, suggesting that they arose from closely related mothers.

The single sampled pony having haplotype 9 descended from matriline 76003, a line accepted into the Stud Book based on phenotype evaluation rather than recorded pedigree. Haplotype 9 is separated from the next closest haplotypes by 10 mutations (haplotypes 7 and 5), which are themselves connected with five mutations between them so it is the most distinct. The pony is known to be the one of only two individuals currently of breeding age descended from that matriline in the UK Exmoor pony population, this founder line is therefore under-represented in the extant population and at risk of extinction unless steps are taken to ensure that it is actively bred from.

Variation of mtDNA haplotypes within extant herds also indicated variation in diversity within herds. Herd 900, the Anchor herd, which contains the highest number of matrilines represented (8), had only three haplotypes: the majority of individuals sampled were haplotype 7, with one individual each having haplotypes 1 and 9. This suggests that most of the matrilines within this herd were closely related, at least on their maternal side, and that the Anchor herd have actively retained most of their foundation matrilines within the herd. Herd 49 had the most ponies from matrilines 12011 and 76001. All except one of the haplotype 2 ponies sampled came from Herd 49, indicating a strong emphasis on breeding from the 76001 matriline within that herd, and that that matriline is not widely dispersed into other herds. Herd 21 had the most 1020 matriline ponies, but these were also found in three other herds (Herds 12, 49, and 78) indicating that this matriline is well dispersed within the UK population. Matriline 12011 was most widely dispersed, found between six herds (Herds 108, 320, 335, 36, 479 and 49).

To reduce the risk of extinction posed by disease outbreaks and other area-specific problems, matrilines and mtDNA haplotypes should be dispersed between geographically disparate herds, although due to the breeding policies of individual breeders this may not always be possible. For example, all the sampled ponies with haplotype 4 were bred in Herd 23, located on Exmoor, and an investigation into the location of ponies with pedigrees likely to have this haplotype, during the selection of individuals for sampling, indicated that within the UK few are bred away from Exmoor. There are, however, likely to be numerous ponies breeding in Europe, especially Germany, carrying haplotype 4 which lowers the risk of their extinction due to UK localised challenges, thanks to their increased geographical dispersal (Exmoor Pony Society Stud Book). This investigation into the mitochondrial haplotypes has thus improved the identification of Exmoor pony matrilines as identified from the stud book, highlighted some inconsistencies in the recorded data and should improve the accuracy of information for breeders. This information may be of value to breeders when they are planning management strategies, as some may choose to concentrate their breeding efforts on the less well represented lines/ haplotypes to reduce the risk of further loss of matrilines.

The Gene Bank Project will expand to include cryogenic preservation of ova and embryos in the future when funding becomes available, and the mtDNA findings from this study will be used in the female donor selection process, so that the full diversity of mtDNA haplotypes can included amongst the preserved samples. This will ensure that the maximum matrilineal genetic variation is preserved, and thus improve the potential viability of any future population resulting from these samples.

Y-chromosome Analysis

Since this study has shown that in common with other horse breeds, there is little diversity in their Y- chromosomal sequences (Wallner et al. 2013; Cardinali et al. 2022), future selection of stallions for the Gene Bank Project will prioritise other considerations; e.g. whole pedigree and physical conformation rather than the concentrating on founder patriline. However, the descendants from the two individuals that clustered separately from the rest of the Exmoors along PC1 and PC2 could make good candidates to investigate further (unfortunately those individuals_are deceased). Unfortunately, the lack of variation in the Y-chromosomes did not permit resolution of some historic unresolved controversies in the pedigree of Exmoors.

Biparental Pairwise Relatedness

An investigation of the significant differences in Pi-Hat relatedness between herds where three or more individuals has been sampled showed no significant differences between herds 21, 49, 479 and 900 (Anchor). However, there were significant differences between herd 23 and all the other herd tested. Herd 900 was the original herd on Exmoor; it is recorded that Herd 1 and Herd 12v foundation stock was originally sourced from the Anchor herd (National Pony Society Stud Book, Exmoor Pony Society Stud Book). Currently, Herd 21 is dominated by Herd 1 foundation lines. Herd 49 and Herd 479 are strongly based on Herd 12 foundation lines. These findings would therefore support the assertion that the Anchor herd, Herd 1 and Herd 12 share common origins. Further study is required to investigate the relationship between Herd 23 and the other herds.

Inbreeding based on ROH

Interpretation of patterns of inbreeding is more informative based on genome-wide patterns of shared ancestry (i.e. runs of homozygosity, ROH). The results of the investigation into ROHs in the Exmoor pony population showed that when compared with 23 other breeds showed that at the 100Kb FROH values (i.e. historical inbreeding, estimated as 500 generations) there were few significant differences between them and the other breeds. The generation interval in horses is usually estimated to be between eight to ten years (Hamann and Distl 2008; Faria et al. 2018). This estimates that the 100Kb FROH values (i.e. historical inbreeding, estimated as 500 generations) is between 4000 and 5000 years ago, around the time that horses were first being domesticated, before the development of modern horse breeds (Gaunitz et al. 2018; Outram 2023). It is therefore not surprising that there are few significant differences between the values at this point.

This changed so that at the 1Mb FROH values (i.e. historical inbreeding, estimated as 50 generations), approximately 400-500 years ago. Exmoors were more distinct from the other breeds, having a significantly higher level of inbreeding than nine of the other breeds. Perhaps at this point the Exmoor pony population was reduced, and this led to an increase in inbreeding. For the most recent inbreeding, 10Mb FROH values (i.e. historical inbreeding, estimated as 5 generations), approximately 40-50 years ago, Exmoors were still significantly more inbred than six other breeds. This suggests that the known population bottleneck in the 1940s increased inbreeding. Numerous studies suggest high levels of inbreeding may result from small founder populations, recent genetic bottlenecks, or genetic drift in a sub-population (Wójcik et al. 2009; Der Sarkissian et al. 2015; Signer-Hasler et al. 2023; Quinn et al. 2023).

Genetic Load in Relation to Inbreeding

The results of this study show a reduction of loss of function alleles from the sampled Exmoor population in relation to inbreeding (R=-0.29, p= 0.03), across all the maternal haplotypes, with a smaller, but not significant in missense alleles (R=-0.18, p=0.19). These results suggest that there is significant purging of the usually more detrimental to fitness loss of function alleles and a slower purging of the less detrimental missense alleles within the population, which is in accord with the purging mechanism suggested by (Hedrick and Garcia-Dorado 2016). However, although not significant, the proportion of homozygous loss of function alleles tended to increase with inbreeding (R=0.18, p=0.18), as would be expected since increased inbreeding will increase overall homozygosity (Hedrick and Garcia-Dorado 2016).

The significant decrease in loss of function alleles with inbreeding may encourage breeders to continue with their current breeding strategies, as this is consistent with purging of the most serious mutations with inbreeding (Kleinman-Ruiz et al. 2022; Grossen et al. 2018).

Since investigation of historic breeding practices of many Exmoor breeders indicates that there has been long-term relative isolation between sub-populations within the breed, with the foundation lines from the Anchor, Herd 1, Herd 12 and Herd 23 being maintained relatively discreetly from each other within the main herds (Society 1980; Baker 2008), evidence of increased purging of loss of function alleles with increasing inbreeding was an unsurprising result. Hedrick and Garcia-Dorado (2016) states that while purging can reduce the negative effects of inbreeding depression, it requires relatively slow inbreeding, and usually a fitness decline is observed before the purging effect becomes apparent. This is likely to have occurred due to the long-term ongoing isolation of sub populations within the overall Exmoor population for many generations, and to the continuous environmental selection pressures that they are exposed to, ensuring that individuals with reduced fitness are unlikely to breed. Exmoor Stud Book records indicate that occasional introductions of new genetic variation into the sub-populations within the breed, usually by the introduction of a relatively unrelated stallion, while maintaining the founder mare band, has been practiced regularly in many herds, arguably a type of genetic rescue, leading to increased fitness within that sub-population. There are numerous examples of the efficacy of this strategy applied to wild populations, including Florida panthers (Johnson et al. 2010), or Bighorn sheep (Hogg et al. 2006). However, genetic rescue may not always have a long-term beneficial effect. Inbreeding post-genetic rescue may lead to increased genetic load due to expression of previously masked recessive alleles inadvertently introduced into the sub-population (Mellya et al. 2023; Hedrick and Garcia-Dorado 2016). Genetic rescue may also make a population more prone to extinction in the long run, despite a short-term advantage, by swamping locally adapted genetic variation (Hedrick and Garcia-Dorado 2016). A waning of fitness after a genetic rescue event was observed in the Isle Royale wolf population in Canada when a subsequent population decline led to the increasingly inbred individuals having lower survival and reproduction rates (Hedrick et al. 2014).

High levels of homozygosity in a population, that has accumulated over many generations, perhaps with associated purging, is not necessarily associated with reduced fitness, an example of this being the Chillingham cattle (Williams 2016, Hall 1988). The Chillingham cattle from Northumberland in northern England, have a 350-year history of complete isolation, with no selective breeding, and consequently high inbreeding, but no observed reduction in viability or fecundity (Williams et al. 2016; Hall and Hall 1988). A study using 777K SNPs indicated that in commercial cattle breeds 62–90% of the SNPs were polymorphic, compared to 9.1% in the Chillingham herd, but that the Chillingham cattle were heterozygous at many of these polymorphic loci, indicating balancing selection at these loci, which are frequently associated with recessive lethal mutations in cattle (Williams et al. 2016). This indicates that these heterozygous regions may be the locations of genes relevant to fitness or survival, which have been naturally selected in the Chillingham cattle over many generations (Williams et al. 2016). A similar study investigating the presence of genes relevant to fitness or survival by comparing Exmoor ponies with other breeds under artificial selection might help to elucidate the genetic basis of the Exmoor's hardiness adaption traits that enable them to thrive under harsh environmental conditions.

Parasite Loads in Relation to Inbreeding

Although only a small subset of individuals had data available on parasite loads, the significantly positive correlation between inbreeding and FEC in a population of Exmoors that are free ranging in the Scottish Highlands (although the most inbred individual was an outlier with no detected parasite load), suggests that despite evidence for purging, there still could be health risks associated with inbreeding. However, an ability to survive and thrive under harsh environmental conditions is why Exmoor ponies are used extensively in rewilding and ecological restoration projects (Hagstrup et al. 2020; Tree 2019). In the UK, Juliet Rodgers, Chairperson of Moorland Mousie Trust, manages 55 conservation grazing sites, home to >200 Exmoor ponies (personal email communication; 22/06/23); Jayne Chapman, Hampshire and Isle of Wight Wildlife Trust, manages numerous Butterfly Conservation and Wildlife Trust sites using Exmoor ponies (personal communication, 28/06/23); Hans Hovens, Chairman Samenwerkingsverband Exmoorpony, reports that there are 602 Exmoor ponies used on conservation sites by 16 organisations in the Netherlands (personal communication, 16/5/23) and many of these sites do not permit the use of anthelminthic treatments for the grazing animals. Thus, it is possible that Exmoors can tolerate high parasite loads, as suggested in chapter 3, which found no apparent association between body condition and parasite loads in the Scoraig Exmoors. This in contrast to a study of Sable Island feral horses, which found that body condition and parasite loads were negatively correlated (Debeffe et al. 2016), which could suggest lower tolerance to parasitic nematodes than in the Exmoors. Nevertheless, the moderate heritability (h2 =0.43, +/-0.11) of parasite loads based on the Sable Island populations (Gold et al. 2019), suggests that selection of stock for use on rewilding sites where there is minimal management intervention could use relative parasite loads as one of the criteria. Further investigation of the relationship between inbreeding, parasite loads and fitness, using a larger sample group and including further investigation of the pedigrees, might offer insights into appropriate selection of individuals with reduced likelihood of parasite-related welfare concerns for use in rewilding situations.

Implications for Exmoor Breeding Strategies

Mean kinship, the average relationship of an animal with the entire population, the mean of all of the kinship coefficients for each animal within a population, is widely used as a tool for avoiding inbreeding depression in managed populations (Ballou, Gilpin, and Foose 1995). An individual with low mean kinship value and therefore which is more genetically diverse from the population average, is considered to be of higher breeding value than one with a high mean kinship value (Ballou, Gilpin, and Foose 1995). For example, the Cleveland Bay horse, a rare breed from the north of England, with pedigree records for 36 generations, has recently been managed using the Rare Breeds Survival Trust's (RBST's) Sparks management tool (https://www.rbst.org.uk/blog/sparks-a-toolbox-for-breeding#:~:text=The%20output%20of%20SPARKS%20is,owners%20when%20selecting%20prospective%20stallions.), which aims to control the rate of increase in inbreeding (Dell et al., 2020 and maximize the effective population size based on selecting individuals to breed based on mean kinship (Dell et al. 2021). Dell et al. (2021) states that its use has increased effective population size, which was previously considered to be unviable, and thereby improved the security of the breed. However, mean kinship, which is aimed at slowing inbreeding and genetic drift in managed populations, will hinder purging since the selection of breeding stock is by low mean kinship value, independent of its fitness, thereby potentially reducing the effect of natural selection (Hedrick and Garcia-Dorado 2016).

The Sparks program is available for use by Exmoor breeders, but uptake has been limited. Cleveland Bay horses have always been kept as a domestic breed with more intensive management than Exmoors. Due to their intensive management their pedigree records are more extensive than those of many other breeds. Exmoor ponies, in their free-living, relatively discrete sub-populations, are continuously exposed to environmental challenges that other, more intensively managed breeds are sheltered from. Purging of seriously deleterious alleles has been found in the sampled Exmoor ponies, likely influenced by these environmental challenges. Their hardiness and ability to thrive unassisted in harsh conditions where other breeds would not is tremendously valued by breeders, who are reluctant to risk compromising these traits by changing their breeding management strategies.

4.10 Conclusion

Investigation of pedigrees during this study indicates that observational records of maternal pedigree have usually been accurate, despite most foals being born in free-living herds. The investigation of relationship between mtDNA haplotypes and founder matrilines found few anomalies: observation of foals with their dams, as with lambs with ewes in Soay sheep studies, usually being a reliable way to report maternal parentage (Hunter et al. 2019; Bérénos et al. 2014). The EPS's verification of parentage using DNA also undoubtedly improves this accurate recording and is especially important for sire verification in free-living herds.

In a rare breed, where the population has been shown to have lost further founder matrilines since the genetic bottleneck in the 1940s, accurate pedigrees are important to inform breeders who wish to use targeted breeding to prevent further erosion of the matrilines. Wójcik et al. (2009) reports a planned management strategy for the preservation of the rare mtDNA haplotypes present in populations of European bison to prevent further loss post-bottleneck. Some Exmoor breeders have already been targeting under-represented matrilines, using pedigree information. This should be encouraged, but using the mtDNA information from this study could be refined, to target increased retention of haplotype diversity. When the Gene Bank project is expanded to include cryogenic storage of ova and embryos the MtDNA information can be used to ensure a range of different haplotypes are included in the stored samples. The Gene Bank's planned cryogenic storage of semen samples is already using information from this study in guiding stallion selection (https://exmoorponysociety.org.uk/the-exmoor-pony-society-gene-bank-campaign/).

It has often been suggested that Exmoors are an ancient breed, little changed from the primitive 'Universal Pony' (Baker 2008; Schafer 1981; Speed and Speed 1977). mtDNA studies have been very widely used in studies of the origins of populations (Librado et al. 2016; Goto et al. 2011; Sharma et al. 2009; Wójcik et al. 2009; Helgason et al. 2000). An exciting area of further study would be a comparison, using molecular methods, of the modern Exmoor population with carbon-dated ancient samples, including those of UK and Western European origins, to investigate the validity of these assertions.

The effective breeding population has been declining since a peak in 2009-2010, now being reduced to levels similar to those in the 1980s. While this is likely to be correlated with reduced numbers of foal registrations, in turn likely to be due to reduced demand, breeders should be encouraged to plan matings carefully when they are reducing the overall numbers of foals produced, to ensure they breed from as wide a range of founder lines as possible. Founder representation across the breed is uneven, and breeders' efforts to breed from under-represented lines should be supported to prevent further losses of founders. Equalizing founder representation is a recommended practice for maintaining captive populations derived from a small number of founders that contribute unequally (Loebel et al. 1992).

An interesting finding that would definitely merit further investigation was that the comparison of Pi-Hat relatedness between the current herds found that one herd, Herd 23, was significantly different to the other herds. This study also found that the mtDNA haplotype 4 was unique to Herd 23 matrilines. Records show that the other herds share a common founder origin, the Anchor herd, unlike Herd 23's foundation stock (Society 1980; Society). Herd 23's geographical origin and breeding management are discrete from the other herds (Baker 2008). An investigation into this difference and its relevance to phenotype could be very interesting in understanding overall variation in the population.

The Exmoor pony population, despite a genetic bottleneck in the 1940s that using ROHs as a measure of inbreeding, for the most recent inbreeding, 10Mb FROH values (i.e. historical inbreeding, estimated as 5 generations), approximately 40-50 years ago, Exmoors were still significantly more inbred than six other breeds, but not significantly different from the 17 others that it was compared with. This means that relative inbreeding had improved when compared to the 1Mb FROH values (i.e. historical inbreeding, estimated as 50 generations), approximately 400-500 years ago, when_they had a significantly higher level of inbreeding than nine of the other breeds. This suggests that recent efforts by breeders have ensured that the inbreeding rate has at least not accelerated in recent generations.

The finding of the reduction of loss of function alleles from the sampled Exmoor population in relation to inbreeding, across all the maternal haplotypes, is suggestive of purging of the most serious mutations with inbreeding (Kleinman-Ruiz et al. 2022; Grossen et al. 2018). Most Exmoor ponies are still bred in free-living herds and thus exposed to continuous environmental selection pressures. This is likely to continue the selection of natural survival fitness traits, which is important if, as this study has found, many Exmoor ponies will be used in low management restoration and rewilding projects. Therefore, further investigation into, and monitoring of the deleterious allele load, and the relationship between parasite load and inbreeding revealed in this study would be of particular value in the context of the Exmoor pony population and rewilding.

Chapter 5: Discussion

Few scientists now doubt that we are facing an unprecedented climate and biodiversity crisis of such severity that without dramatic action there will be rapidly increasing global suffering for all life on Earth (Ripple et al. 2023). Previously, ecological restoration has had a paradigm of re-establishing ‘reference' communities that conserve ecosystems as they were; however, in the face of rapid environmental change this may not create sufficiently resilient, adaptable, and complex communities (Bullock et al. 2022). Humans have impacted nearly the whole surface of the planet, yet to retain biodiversity there must be space for other species (Perino et al. 2019).

Rewilding is an approach which, specific to each ecosystem, aims to mitigate against the influence of humans from anthropogenically altered ecosystems (Perino et al. 2019). Trophic rewilding advocates the re-introduction of keystone species, often large herbivores, to create a top-down cascade of effects creating self-sustaining, functional ecosystems (Perino et al. 2019). The aim of rewilding is thus the restoration of wildness, rather than wilderness (Perino et al. 2019).

Rewilding Europe have an admirable mission statement ‘We want to demonstrate the benefits of wilder nature through the rewilding of diverse European landscapes, and to inspire and enable others to engage in rewilding by providing tools and practical expertise' (https://rewildingeurope.com/wp-content/uploads/publications/rewilding-horses-in-europe/index.html). They currently support eleven projects across Europe using keystone herbivores to initiate top-down trophic interactions with their associated trophic cascades to promote them becoming self-regulating biodiverse ecosystems. They widely use horses as part of the process and have published guidelines stating that as the ancient European wild horse became extinct, they advocate the substitution of hardy, independent free-living breeds that have retained ancient, wild genetic traits and natural behaviours similar to the Exmoor or Konik breeds (https://rewildingeurope.com/rewilding-in-action/wildlife-comeback/wild-horses/). They state that the animals will show the proof of their suitability for rewilding by thriving in practice. However, they indicate that their goal is not to keep pure-bred locally adapted animals but that after a process of rewilding, the foundation breeds will no longer be purebred, but will gradually change to rewilded horse types. Their aim is to create a genetically diverse population with improved fitness which will become the rewilded horse type. Rewilding Europe state that while in the short term this may impact negatively on the welfare of some individuals, it will ultimately result in improved fitness for the overall population. They acknowledge that some individual breed societies may oppose this idea, and there will definitely be opposition from many Exmoor breeders.

There is a parallel in this with the ideas advocated by Lutz and Heinz Heck, amongst others, concerning the 'back-breeding' of cattle to re-create extinct aurochen (Driessen and Lorimer 2016). The Heck brothers sought to resurrect the aurochen by breeding out what they perceived to be the degeneration associated with domestication, mixing multiple breeds to re-create primitive animals unadulterated by human influence, (by selecting traits they identified as 'pre-domestication traits' from multiple breeds, and cross-breeding these selected animals) part of the National Socialist ideology of ethnic purity as a form of ecological restoration, partially used in justification of the Nazi eastern expansion during WW2 (Driessen and Lorimer 2016). Leaving aside the political ideology behind this attempted back-breeding, the attempt was not entirely successful, there are only a few descendants of the resulting stock left, and appropriately for today, hardy cattle breeds are widely and successfully used in rewilding; e.g. Longhorns at Knepp in the UK;(https://knepp.co.uk/rewilding/free-roaming-herbivores/longhorn-cattle/), or Galloways at Mols in Denmark (https://www.naturhistoriskmuseum.dk/mols-laboratory/a-wilder-mols-laboratory). Another example is the Chillingham cattle, a closed population for hundreds of years, and described as 'the only wild cattle' continue to thrive without human intervention, but not technically rewilded as they have arguably never really been domesticated (https://chillingham-castle.com/wild-cattle/). Painted by Landseer and eulogised by Sir Walter Scott, they are a cultural icon (Ritvo 1992). Cross-breeding of such breeds could compromise the traits that have made them persist without intervention.

As my study has shown, Exmoor ponies have many positive traits, including: their grazing habits; their possible avoidance or tolerance of parasite load so that their body condition can be successfully seasonally regulated; their possible purging of seriously deleterious alleles, and their ability to thrive under minimal intervention management, possibly due to retention of primitive traits, making them suitable for use in conservation, restoration and rewilding projects. They are already being used in rewilding projects at; Knepp Estate in England, (https://knepp.co.uk/rewilding/free-roaming-herbivores/longhorn-cattle/); Bamff, in Scotland (https://bamff.land/natural-grazing/); Mols, in Denmark (https://www.naturhistoriskmuseum.dk/mols-laboratory/a-wilder-mols-laboratory); and in the Netherlands, overseen by Samenwerkingsverband Exmoorpony (personal email communication, 2/1/24: Nieuwsbrief Samenwerkingsverband Exmoorpony, December 2023). In the UK, the Moorland Mousie Trust has nearly 200 Exmoors conservation grazing at >30 sites in the north of England (personnel email communication from Juliet Rodgers, Chairman Moorland Mousie Trust 28/4/23) and there are many others in the south of England, as well as Exmoors grazing on Wildlife Trust sites (https://www.kentwildlifetrust.org.uk/climate-change/conservation-grazing/exmoor-ponies, https://www.durhamwt.com/how-help-exmoor-ponies, https://www.hiwwt.org.uk/news/exmoor-ponies-help-isle-wight-rewilding-project). By crossing them with other breeds there is a risk of losing the adaptation traits that have made them survive with little intervention, the hybrids usually not having the same fitness under harsh environmental conditions as the pure-bred Exmoor ponies (Hovens 2014; Baker 2008). The Samenwerkingsverband Exmoorpony, who oversee organisations using >600 pure-bred Exmoors on ecological restoration sites, includes a report of a site where Exmoors have sometimes cross-bred with Koniks, indicates that these hybrids are less suitable, and their breeding should be discouraged. Rewilding Europe's publication includes a map showing the geographical range likely to be appropriate for specific European horse breeds (https://rewildingeurope.com/rewilding-in-action/wildlife-comeback/wild-horses/). Local adaption traits of free-living breeds would merit further study to determine what adaptations improve fitness under differing local conditions, for example, what adaptations are particularly relevant in a cold wet cold, dry cold or drought prone environment. This could improve the selection of suitable stock in differing climatic regions. Breeding herds of rare breed animals on restoration/ rewilding sites, would not only benefit the site through their ecological interactions with other species present in the habitat, but would contribute to the maintenance of biodiversity through protection of the future population of those animal eco-engineers, and produce more individuals so that surplus stock could be available for transfer to other sites. Rewilding Europe have started a scheme to put managers in need of stock in touch with those who have surplus (https://rewildingeurope.com/news/banking-on-wildlife/), but this co-ordinated stock management is not always practiced. The EPS will try to match enquiries for stock with breeders, and the Moorland Mousie trust supply grazers for conservation grazing, but development of an effectively co-ordinated network for breeders and conservation projects, including appropriately managed animals would undoubtedly be beneficial for helping to safeguard many rare breeds.

An appropriate management background can be extremely relevant for animals kept on large, low-intervention projects, especially where specific behavioural traits may be important. Although Exmoors born into free-living herds can adjust to domestic life with appropriate handling, preferably started at an early age (personal experience), as evidenced by the many Exmoors living successful lives as working or leisure animals in domestic homes, (https://exmoorponysociety.org.uk/), transition from a domestic to a free-living environment could be more challenging, in terms of behavioural adaptations, tolerance to novel pathogens, appropriate food-source seeking or hardiness to adverse weather conditions.

A very specific example of the relevance of the retention of ancestral behavioural traits becomes apparent when ponies and large carnivores co-exist in a habitat. Trophic rewilding advocates the reintroduction of missing keystone species, including large carnivores (Perino et al. 2019). It often uses re-introduced species as ecological proxies for species that became extinct centuries or millennia ago (Perino et al. 2019). Where these species have not retained the behavioural traits that include defence against predators this could lead to unacceptable losses; e.g. <70% of Garrado foals are lost to wolves in some scrubland areas of northern Portugal (Santos and Ferreira 2017). Exmoor ponies have been bred in a wolf-free environment in Western Europe for many generations; the last wild wolf in the UK was probably killed in Scotland in the mid 18th century (https://www.wolvesandhumans.org/history-of-the-wolf-in-scotland). Exmoors kept in the Netherlands are now naturally coming into contact with wolves and apparently, although there have been losses of a few immature males, many retain the defensive instincts to protect themselves and their foals (Nieuwsbrief Samenwerkingsverband Exmoorpony, December 2023). This could represent the re-surfacing of a long dormant trait in the presence of an appropriate stimulus and is relevant to the future of Exmoors on rewilding projects. Further study to investigate whether the behaviour is widespread or confined to specific bloodlines within the breed, and whether naïve groups could learn the behaviour from experienced animals would be of interest.

Another area that requires further investigation is the attitude of the public to carnivore/ herbivore interactions. Currently the ecological functions provided by large carnivores in Europe are largely relegated to remote areas, with relatively little awareness by the public about the direct consequences of carnivore/ herbivore interactions (Davoli et al. 2023). The current reduction in European megafauna in comparison to the past has resulted in a faunal simplification, which has led to woody densification associated with reduced presence of large herbivores in the landscape in some regions, while conversely, in other regions there is loss of biodiversity linked to overgrazing by the high populations of deer, which in turn is likely to be linked to reduced or absent large carnivore assemblages (Davoli et al. 2023). As large carnivore populations increase, or as carnivore species are re-introduced into regions formerly part of their range, the attitudes of the human communities that will interact with them need to be considered (Davoli et al. 2023). Whether the urbanised public in Europe will accept the natural consequences of carnivore/ herbivore interactions if they witness them first hand (for example, finding the remains of a predated foal whilst enjoying a trip to a natural environment) remains to be seen; however, rewilding managers may applaud the associated enhanced ecosystem functioning. The relationship between people and the wildness in nature has always been paradoxical and ecosystems cannot be assessed independently from human society (Perino et al. 2019). Education may be key to improving the public's understanding.

A UK government report using from October 2021ahead of the COP26 UN Climate conference in Glasgow indicated that 75% of adults in Great Britain said they were worried about the impact of climate change (https://www.ons.gov.uk/peoplepopulationandcommunity/wellbeing/articles/threequartersofadultsingreatbritainworryaboutclimatechange/2021-11-05). Reports from a broad cross-section of the British press indicate growing public concern over the climate and biodiversity crises, but also reflect their uncertainty about how to act in response to the problems (Table 5.1). The problems are complex and require complex approaches in response to them but complexity is not an easy message to spread.

Planting three billion trees to capture carbon and seeking measures to reverse pollinator decline are two key pledges of the EU Biodiversity Strategy for 2030 (Pérez-Gómez, Godoy, and Ojeda 2024). Simplistic messages often receive enthusiastic responses, the 'plant a tree and help to save the world' message is very appealing (http://www.onetreeperchild.com/#schools, https://www.justonetree.life/, https://www.treeappeal.com/Schools.html), but not always followed through in an appropriate manner. It is possible to pay to have trees planted to offset the carbon footprint from flights or from businesses (Table 5.2), but these offsetting schemes although superficially appealing due to their simple message, are not always effective carbon sequestration management (https://www.theguardian.com/environment/2022/mar/05/lost-forest-why-is-brewdog-green-scheme-causing-controversy). Tree planting can be an effective carbon store, but not in every situation, and in previously un-wooded ecosystems planting can have negative consequences for pollinators and other invertebrates (Pérez-Gómez, Godoy, and Ojeda 2024). Explaining the importance of the biodiversity and of the carbon sequestration potential of grasslands and peatlands to the public is a less obvious and more complicated process, but one that is interlinked with rewilding and must be fully engaged with (Bai et al. 2022; Alexandrov et al. 2020; Dass et al. 2018; Leifeld and Menichetti 2018).

Rewilding areas have inevitably been subjected to human influences so management of the interaction between people and those ecosystems is crucial to increase the acceptance and success of rewilding projects (Perino et al. 2019). People enjoy exposure to nature and exposure to charismatic or symbolic species or landscapes can be inspiring, promote spiritual well-being and be part of cultural heritage (Perino et al. 2019). People love to visit sites where they can see ecological restoration in action (https://www.thetimes.co.uk/article/why-the-knepp-rewilding-project-is-truly-magical-m68trp899). However, there has not always been a positive trajectory towards acceptance of rewilding, because the natural processes of death and predation are not always pleasant to observe. The highly publicised deaths of starving animals at the Oostvaardersplassen (OVP) rewilding project in the Netherlands in 2016-2017 led to policies based on the Van Geel Commission report for active population control of the ungulate populations, and subsequently to debates as to whether this undid the genuine ‘rewilding’ aspect of the project, as it increased human intervention (https://rewilding.org/european-experiments-in-rewilding-oostvaardersplassen/). OVP had no carnivores to control the ungulate population, neither were there corridors allowing migration between different areas, so arguably human ‘predation’ (removal) of animals to maintain stocking density at the expected carrying capacity of the area, thus mimicking natural processes.

Another area of potential conflict is where people perceive rewilding to be exclusive of them, where it is perceived, that people should be entirely removed from large areas, and where it feels as if there is an ‘erasure’ of human traditions and cultural involvement with the land (Perino et al. 2019). Farmers may see rewilding as an undermining of their roles, an erosion of their contributions to food security and the destruction of the rural landscape as they have shaped it. When rewilding projects and the values of local farmers are in opposition there may be social conflicts; dialogue and social engagement is required to mitigate these tensions (Mikołajczak et al. 2022). Farmers fear loss of their livelihood (https://www.theguardian.com/environment/2021/dec/28/agriculture-recycling-carbon-farmers-reframe-rewilding-debate), that rewilding will affect food security (https://www.telegraph.co.uk/business/2023/07/12/race-to-rewild-britain-is-putting-food-security-at-risk/, https://www.bbc.co.uk/news/articles/c892vvwr2gpo, ‘you can’t eat trees’), or undermining traditional rural systems (https://www.fwi.co.uk/news/opinion-beavers-spark-guffaws-and-confusion, ‘We’ve managed without ’em fer 400 years and there ain’t nothing wrong with our countryside!’ on the introduction of beavers to Knepp Estate).

Reconciling these opposing views requires communication and education. The Food Sustainability Index (FSI), indicates that in 2018 the UK ranked 55th out of 67 countries on sustainable agriculture (https://impact.economist.com/projects/foodsustainability/blogs/can-rewilding-and-farming-be-combined-to-build-a-better-food-system/). Farming uses 71% of UK land, while rewilding is proposed for 5% of the land, with 25% of land to be used for more nature-friendly forms of farming, forestry (https://impact.economist.com/projects/foodsustainability/blogs/can-rewilding-and-farming-be-combined-to-build-a-better-food-system/). Knepp Estate is an example of a successful hybrid model of farming and rewilding, where the decision to change the land uses from unprofitable conventional farming to a rewilded area that is profitable from ecotourism and from sales of organic meat for economic reasons (https://impact.economist.com/projects/foodsustainability/blogs/can-rewilding-and-farming-be-combined-to-build-a-better-food-system/).

The future of rewilding and the future of the Exmoor pony population appear to be strongly entwined. In the UK, most Exmoor ponies are born into free-living herds even though for many their eventual destiny is to live in a domestic situation. In Europe, the use of Exmoor ponies in rewilding will mean that a large proportion of the global population will be born into free-living herds, where they will continue to be subject to natural, environmental selection pressures which will in turn favour the retention of the survival and positive ecological interaction traits that this study indicates that they have retained: their grazing habits that have a positive effect on biodiversity, and especially flowering plant abundance; and their ability to efficiently regulate body condition seasonally and their toleration of parasite loads. This continued environmental selection pressure on fitness also suggests that the purging of deleterious traits found in this study is likely to be on-going, and would merit continuous monitoring, so that if on future investigation a trend towards increasing frequency of deleterious alleles was found, informed action could be implemented to reduce harm to the breeding population.

In free-living herds identification of a foal's dam should be relatively straightforward for observers, and this study has shown that the pedigree records of matrilines and the mitochondrial evidence has mostly been remarkably congruent. Without DNA parentage verification, however, identification of patrilines, especially in herds where multiple males are present, is more challenging. Investigation of the post-genetic bottleneck female and male founder lines has shown that their representation in the extant population is uneven, and that erosion of genetic diversity through the extinction of some founder lines has occurred. The representation of some founder lines is such that their future extinction is possible if prompt action is not taken to maintain them. In the past breeders have often responded positively to information about under-represented bloodlines and have taken steps to redress possible losses of founder lines, so ongoing monitoring and dissemination of the results may be effective in preventing further extinctions. The effective breeding population has fluctuated over time and is currently showing a declining trend. While this may be associated with reduced overall breeding production, in response to the decreased market demand for foals, careful selection of brood stock can mitigate the negative effect on the effective breeding population. Improving breeders' awareness of the representation of the founder lines within the breed, combined with DNA parentage verification to ensure accurate pedigrees, can help to reduce the risk of further loss of founder lines. The EPS's Gene-Bank Project aims to ensure the cryopreservation of semen from as wide a range of Exmoor stallions as possible is another emergency safeguard for the breed; results from my study are already being used to inform stallion selection through the identification of under-represented founder lines (https://exmoorponysociety.org.uk/the-exmoor-pony-society-gene-bank-campaign/). Technical limitations and expense have so far limited the cryogenic biobanking to the storage of frozen semen, but in the future storage of ova, embryos and tissue would further enhance safeguarding of the future population. The mapping of mtDNA haplotypes to founder matrilines in this study could inform the selection of candidates for ova storage to ensure the maximum possible haplotype diversity is represented in the chosen samples.

The conservation of single species in isolation does not work. Exmoor ponies must have a role to survive in the future and linking that role to the preservation of their habitat will increase their own conservation value. As this study has shown, Exmoor ponies in conservation and rewilding play an important role in positively influencing biodiversity and flowering plant abundance in grasslands, but they may also have a role in climate mitigation. For example: 1) grazing has been shown to stimulate the reallocation of non-structural carbohydrates to root storage in oak trees, thus improving below ground carbon sequestration; and 2) at appropriate densities in rewilding projects grazing can promote natural tree colonisation, positively impacting wood-pasture succession, ecosystem function and complexity (Burrell et al. 2024). Rewilding projects can therefore contribute both to biodiversity gains and to climate change mitigation (Burrell et al. 2024). This role may be crucial to their survival in a world threatened by the crises of biodiversity loss and climate change.

Arguably, in the same way that Chillingham cattle are held up as a 'cultural icon' (Ritvo 1992), perhaps Exmoor ponies should be viewed in the same way. Schafer (1981) stated that he believed them to be the nearest remnant population to the ancient Universal Pony that colonised Western Europe after the Ice Age. Morphological studies showed their skulls, teeth and long bones to closely resemble those of >10,000-year-old equine remains found in caves in Southern England (Speed and Speed 1977). They have appeared in literature described as the native wild ponies; their independence remarked upon since the publication of R.D. Blackmore's novel 'Lorna Doone' in 1869. The ponies are a key attraction in encouraging tourism in the Exmoor National Park (https://www.nationalparks.uk/park/exmoor/). The depictions of the Celtic-Roman horse goddess Epona usually depict her surrounded by small, thick-set Exmoor-like ponies (Figure 5.1). Further studies, using molecular methods to compare modern Exmoors with ancient remains to investigate their ancestry and their relationship to other north-western European horse breeds could be used to elucidate the validity of claims of their ancient origin and also to investigate the influence of Exmoors in the history of modern horse breeding.

Figure 5.1 Roman period images of the Celtic-Roman goddess Epona surrounded by small, thick-set Exmoor-like ponies (Maup van de Kerkhof, "Epona: A Celtic Deity for the Roman Cavalry", History Cooperative, January 20, 2023, https://historycooperative.org/epona/. Accessed January 23, 2024).

Table 5.1 British press reflects public concerns over climate change and biodiversity loss

Date	Newspaper	Author	Headline	Link
7/11/22	The Guardian	Roger Harrakin	Why Scientists are using the word scary over the climate crisis	https://www.theguardian.com/environment/2022/nov/07/why-scientists-are-using-the-word-scary-over-the-climate-crisis
14/7/23	The Guardian	Jonathan Freedland	As heat records break, the climate movement...	https://www.theguardian.com/commentisfree/2023/jul/14/big-oil-climate-crisis-fossil-fuel-public
30/3/23	The Times	Kat Ley	Climate change is the biggest long-term risk to human health	https://www.thetimes.co.uk/article/climate-change-is-biggest-long-term-risk-to-human-health-say-doctors-sgpx6ch2g
11/10/22	The Mirror	Sarah Lumley	Most brits are desperate for Government to take more climate action...	https://www.mirror.co.uk/news/uk-news/climate-change-nature-government-action-28200736
28.2.22	The Mirror	Nada Faroud	Climate change hits billions...	https://www.mirror.co.uk/news/uk-news/breaking-climate-change-hitting-billions-26346955
8/9/23	The Telegraph	Matt Ridley	The public isn't being told the full truth about the climate...	https://www.telegraph.co.uk/news/2023/09/08/the-public-isnt-being-told-the-full-truth-about-the-climate/
29/11/23	The Guardian	Patrick Greenfield	A biodiversity catastrophe...	https://www.theguardian.com/environment/2023/nov/29/a-biodiversity-catastrophe-how-the-world-could-look-in-2050-unless-we-act-now-aoe
12/7 /22	The Times	Jonathan Baddash	Biodiversity crisis could threaten human existence	https://www.thetimes.co.uk/article/biodiversity-crisis-could-threaten-human-existence-rswlhp0q3
13/10/22	The Mirror	Nada Faroud	Life on Earth on the brink of catastrophe	https://www.mirror.co.uk/news/world-news/one-million-species-risk-extinction-28223916

26/7/23	The Mirror	Nada Faroud	Climate change will wipe out almost 800 species	https://www.mirror.co.uk/news/world-news/climate-change-wipe-out-almost-30561961

Table 5.2 Carbon offsetting for flights and businesses

Carbon offset purpose	Link
Offset your flights	https://onetreeplanted.org/products/carbon-offset?g_adtype=&g_network=g&g_keyword=carbon%20offset%20programs&g_placement=&g_campaignid=10867721200&g_adid=661276705364&g_merchantid=&g_ifcreative=&g_locphysical=9046941&g_source=%7Bsourceid%7D&g_keywordid=kwd-3168191205&g_campaign=account&g_acctid=611-028-5007&g_partition=&g_productchannel=&g_productid=&g_ifproduct=&g_locinterest=&g_adgroupid=150410266736&gad_source=1&gclid=EAIaIQobChMIgLOTx8HxgwMVkKODBx0NZQRLEAAYAiAAEgKO0_D_BwE%22%20%5Ct%20%22_blank
Offset your business carbon	https://rippleafrica.org/forest-conservation-and-tree-planting/?gclid=EAIaIQobChMIgLOTx8HxgwMVkKODBx0NZQRLEAAYASAAEgIFLvD_BwE
Offset your business carbon	https://carbonneutralbritain.org/pages/become-a-carbon-neutral-business?kw=ga-carbon-offsetting-exact&utm_source=google&utm_medium=cpc&utm_campaign=novi---carbon-neutral-britain---carbon-offsetting---exact&utm_term=carbon-offsetting&gad_source=1&gclid=EAIaIQobChMItqyFpoH7gwMVTaeDBx2sGQtwEAAYASAAEgLbbfD_BwE
Offset your business carbon	https://www.5dnetzero.co.uk/product-category/projects/?gclid=EAIaIQobChMIwLeB2oH7gwMVlpaDBx0O2gB4EAAYASAAEgLIR_D_BwE
Offset your business carbon	https://ecologi.com/beyond-carbon-offsetting?gad_source=1&utm_campaign=EX+-+UK+-+Activation+-+Generic+Text+-+%3CGeneric+Text+-+UK+Core%3E+-+Conversions&utm_medium=cpc&hsa_mt=p&hsa_cam=18503830537&hsa_kw=offset+carbon+footprint&hsa_acc=3450399250&utm_term=offset+carbon+footprint&gclid=EAIaIQobChMI86bs-YH7gwMVaJVQBh2eZQ1REAAYAyAAEgJsdvD_BwE&hsa_tgt=kwd-336158343804&hsa_src=g&hsa_ver=3&hsa_grp=141292897679&hsa_ad=648476254720&hsa_net=adwords&utm_source=google
Offset flights carbon	https://www.klm.co.uk/information/legal/extra-options/co2-impact-programme
Offset flights carbon	https://clear.eco/carbon-offset-my-flight/?gclid=EAIaIQobChMIktXU4YL7gwMVM5JQBh2u2A-LEAAYAyAAEgILuvD_BwE

Appendices

Photo: Dave Maric at Bamff wildlands

APPENDIX 1.1

Table A1.1 Literary sources indicating cross-breeding of British native pony breeds (with thanks to Sue Baker for help in compiling this table).

BREED	SUB-POPULATION	INTRODUCTIONS (multiple literature sources)	INTRODUCTIONS (single literature source only in each case)
EXMOOR	Exmoor Ponies extant population (Anchor herd)	None *	Thoroughbred; Arab*
	Ponies on Exmoor extinct population (Knight)	Thoroughbred; Arab	
FELL		Friesian; Galloway	Dales; Exmoor; Hackney; Highland; Irish capul; Norfolk Cob; Welsh Cob
SHETLAND		Norwegian; Icelandic; (plus Arab on Fetlar)	Hackney; Highland
WELSH MOUNTAIN		Hackney; Thoroughbred; Arab	Connemara; Dartmoor; Eastern; Fell; Polo Pony
HIGHLAND	Mainland	Norwegian; Clydesdale; Icelandic; Percheron	Arab; Fell; Galloway; New Forest; Spanish; Welsh
DALES		Fell; Galloway; Welsh Cob/Trotter; Norfolk Cob/Trotter; Clydesdale; Hackney	Connemara; Friesian; Highland; Norwegian
DARTMOOR		Exmoor; Welsh; Arab; Hackney; Thoroughbred; Cob; Fell	Barb; Danish; Goonhilly; New Forest; Polo Pony; Roadster; Shetland
NEW FOREST		Arab; Dartmoor; Exmoor; Fell; Highland; Welsh; Thoroughbred; Dales; Hackney	Irish; Shetland; Spanish
CONNEMARA		Hackney; Welsh; Spanish; Arab; Thoroughbred; Barb; Clydesdale; Irish Draught; Norwegian	Icelandic; Western Isles

Notes: * Extant Exmoor population – possible introgression in the 1920s when records state that 8 Anchor herd mares were sent to a Fortescue crossbred sire, although no records exist suggesting that any progeny from these matings were retained in the Anchor herd (Baker 2008).

LIST OF LITERATURE SOURCES:

BREED HISTORIES

Sue Baker, *Survival of the Fittest* 2nd edition Halsgrove 2008 (Exmoor Pony)

Wynne Davies *The Welsh Mountain Pony* J A Allen 1993
Iona Fitzgerald *Dales Ponies* Whittet Books 2000
A E Fraser *The Days of the Garron* Macdonald 1980 (Highland Pony)
Anna Hodson *Shetland Ponies* The Crowood Press 1990
Pat Lynne *Shrouded in Mist* self-published 1984 (Connemara Pony)

Dionis Macnair *New Forest Ponies,* Whittet Books 2006
Clive Richardson *The Fell Pony* J.A. Allen 1990
Valerie Russell *Shetland Ponies* Whittet Books 1996

BRITISH NATIVE PONY BREEDS

Daphne Machin Goodall *British Native Ponies* Country Life Ltd. 1963
Valerie Russell *Discovering British Ponies* Shire Publications 1976
Peter Upton *Out of the Desert* Medina Publishing 2010
Robert Wallace *Farm Livestock of Great Britain* 5th Edition Oliver & Boyd 1923

REGISTRATION STUD BOOKS

Polo and Riding Pony Stud Books Volume VII to Volume XII (1903 – 1912)
National Stud Books Volume XIII to Volume XXV (1913 – 1947)

BREED WEBSITES (including relevant information)

https://www.britishconnemaras.co.uk/

https://www.dalespony.org/
https://exmoorponysociety.org.uk/

https://www.fellponysociety.org.uk/
https://www.newforestpony.com/
https://www.shetlandponystudbooksociety.co.uk/

https://wpcs.uk.com/

APPENDIX 2

Maps of the treatment areas at the study sites

Figure A2.1: The treatments at Achmore showing the grazed and exclusion treatments included in the study. Areas outlined in yellow have been continuously grazed by ponies for >30years. The area outlined in red is a previously grazed area that the ponies have been excluded from for the duration of the study. Grazed field identifiers: 1, top west field, 2, bottom west field, 3, middle field, 4 top east field, 5, bottom east field, 6, exclusion field.

Figure A2.2: The inclusion and ‘control’ treatment sites at the Scoraig croft. The area outlined in yellow is the inclusion treatment, an area not previously grazed by ponies to where they were introduced at the start of the study. The area outlined in pink is the ‘control’ treatment site, which is grazed by cattle and sheep but has been un-grazed by ponies for >30 years.

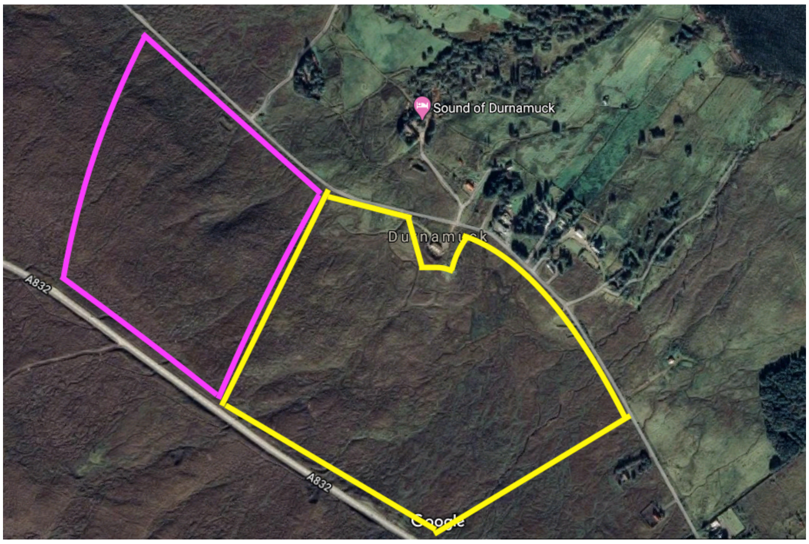
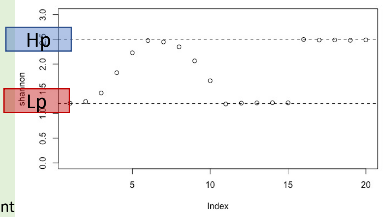


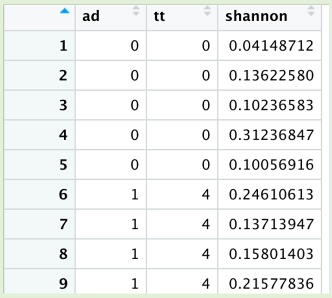
Figure A2.3: The inclusion and exclusion treatments at Durnamuck. The exclusion treatment, an area un-grazed by ponies is outlined in pink. The area to which ponies were introduced in October 2019, the inclusion treatment, is outlined in yellow.

APPENDIX 3

Details of the Bayesian predictive model code.

```
# SIMULATION

Hp<-2.5 # High plateau (asymptote, won't necessarily achieve this, it is long term )
Lp<-1.2 # Low plateau ( baseline from which we may see change)

a0<--4.6 # Baseline coefficient assumes that process starts from 0.01 complete

a1<-1.3 # Rate after addition
a2<-0.2 # addition increment/decrement
T<-2*(-a0)/a1 # Maximum time for equilibrium - assumes process finishes at 0.99 complete
tE<- c(0,1,2,3,4,0,1,2,3,4,0,0,0,0,0,0,0,0,0,0)# Time since treatment applied, the time Exmoors are present/absent
adE<- c(1,1,1,1,1,0,0,0,0,0,1,1,1,1,1,0,0,0,0,0)# Addition Y/N? Exmoors present or Exmoors not present
#tS<- c(0,1,2,3,4,)
n<-length(tE)
te<-(1-adE)*T-(-1)^adE*tE # Travel time in relation to hi e.g.How much
#it goes up  between LP and HP

b<-(a1+adE*a2) # b changes with addition or removal of Exmoor ponies
L<-a0+b*te  # y=a+b*x  : L=a0+a1*t : L=a0+(a1+ad*a2)*t
H<-exp(L)/(1+exp(L))
biod<-Lp+(Hp-Lp)*H
shannon<-pmax(0,rnorm(length(biod),biod, 0.01))

plot(shannon, ylim=c(0,3))
abline(Hp,0, lt=2)
abline(Lp,0, lt=2)
```





	ad	tt	shannon
1	0	0	0.04148712
2	0	0	0.13622580
3	0	0	0.10236583
4	0	0	0.31236847
5	0	0	0.10056916
6	1	4	0.24610613
7	1	4	0.13713947
8	1	4	0.15801403
9	1	4	0.21577836

ad = **ad**d Exmoors (0=no, 1= yes)
Tt = **t**ime since **t**reatment applied)

```
introMod<-"model{

for(i in 1:n)
{
 t[i]<-(1-ad[i])*ttot-(-1)^ad[i]*tt[i]
 L[i]<- -a0+(a1+ad[i]*a2)*t[i]
 biod[i]<-Lp+(Hp-Lp)*exp(L[i])/(1+exp(L[i]))
 shannon[i]~dnorm(biod[i],obsPrec)
}


# Priors

Lp~dgamma(1,10) # Low plateau
dp~dgamma(1,10)
Hp<-Lp+dp # High plateau
a0~dgamma(1,1)
#a0<-4.6
a1~dgamma(0.1,100)
#a1~dnorm(0,0.1)# 1st is mean, 2nd precision (inverse of variance)allows negative as well as positive changes
a2~dnorm(0,0.1)T(-a1,)
ttot<-abs(2*a0/a1)
rate<-a1+a2 # Rate of enrichment, as compared to a1, which is the rate of impoverishment
obsPrec~dgamma(0.1,0.1)
#data# ad, tt, shannon, n
#monitor# a0,a1,a2,Lp,Hp,ttot, rate,obsPrec
#inits# a1, a2


}"
```

APPENDIX 4

Observed effects of trampling and removal of tall vegetation, especially rushes, by Exmoor ponies creating short canopy height lawns which benefit forb abundance.

A B C

The effect on vegetation of introducing Exmoor ponies to a previously un-grazed area:
A: Trampling by ponies created paths though tall vegetation.
B: Exmoor pony grazing reducing the vegetation canopy height.
C: Abundant flowering plants (orchids) in a short canopy lawn.

Appendix 5

Body condition scoring

To estimate overall health of animals, body condition was scored using the Carroll and Huntington (1988) approach, but without physical palpitation as some ponies in the study were unhandled. The method assumes that fat reserves depend on a balance between energy intake and expenditure (Henneke, Potter, and Kreider 1984). A visual inspection was made of the entire animal to assess the amount of fat over the ribs, tailhead and quarters, neck, and withers and spine, to allow the comparison of ponies with differing amounts of stored body fat, (Figure 1) independent of the body size, sex, or age of the pony. A score of 0-5, quantified in evenly spaced intervals of 0.5 between the lowest and highest scores, with 0 representing an emaciated animal with little or no fat cover to 5, an obese animal with heavy fat deposits would then be allocated to each animal. Figure 1 shows the areas of the pony used in the assessment. Figures 2A–2W show examples of Exmoor ponies of different ages and sexes exhibiting body condition scores from 1.5, poor condition to 5, obese. Different ages and sexes of animals assessed to have the same body condition score are included to illustrate the differences in outline that are seen due to sexual characteristics or variations in maturity.

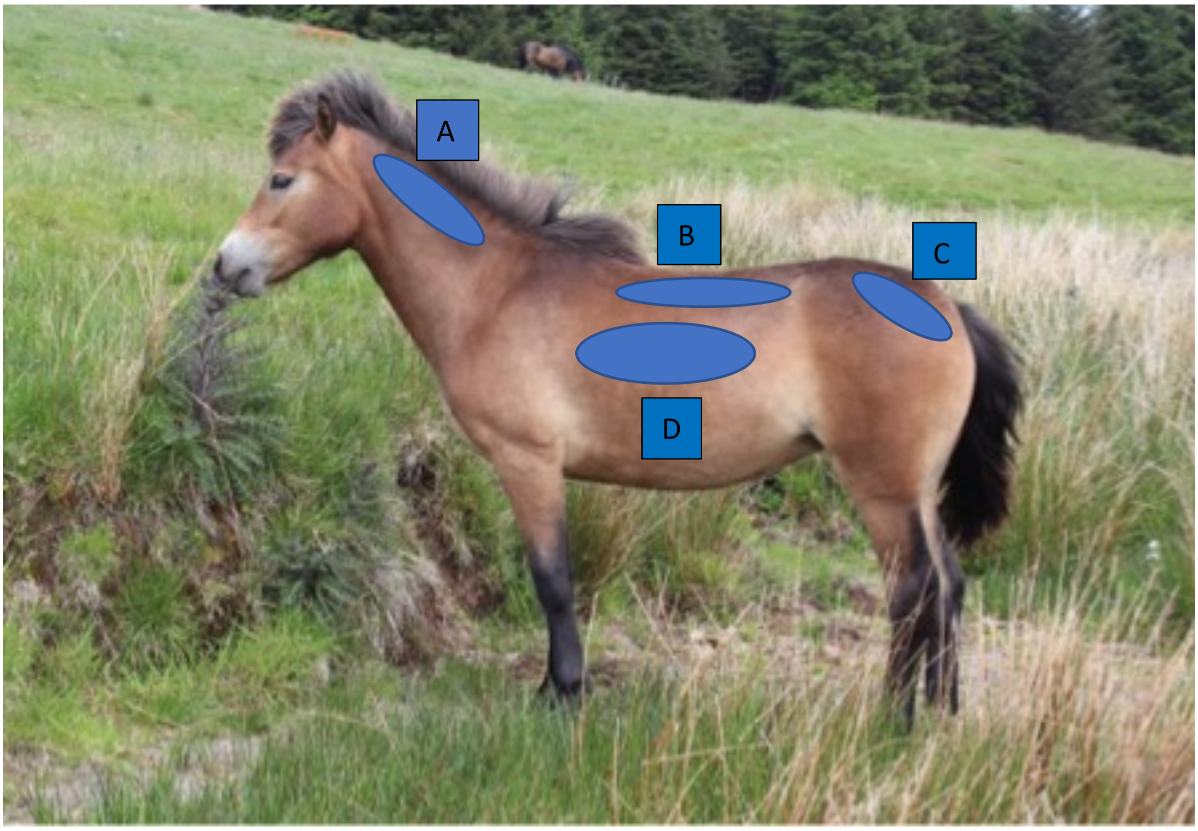


Figure A5.1A: The areas of the pony where fat cover is assessed to give a body condition score. A: Neck and crest, B: Along the spine over the spinous processes, C: Tailhead and hindquarters, D: Cover over the ribs.

Figure A5.2A: Yearlings, A: condition score 1.5, B: condition score 2. Photographed in spring after wintering on hill ground. Ribs were clearly visible, the spines were prominent and there was little fat cover over tailhead, hindquarters and neck.

Figure A5.2B: Yearling, condition score 2. It was difficult to assess body condition score of this animal due to moulting obscuring its outline, but some fat cover on neck, tailhead and hindquarters was observed.

Figure A5.2C: 4-year-old mare, condition score 2. Photographed in spring while her winter coat obscured her outline. Careful observation of her neck and back allowed accurate assessment of her condition, ribs had a light fat cover and were not immediately visible, her neck and hindquarters had some fat cover.

Figure A5.2D: 5-year-old mare, condition score 2.5. Photographed in spring after wintering on hill ground, shedding her winter coat, her outline was clearly visible. Ribs had some fat cover and were not obviously visible. Her spine, neck and hindquarters showed moderate fat cover.

Figure A5.2E: 8-year-old stallion: condition score 2.5. Photographed in spring after wintering on hill ground, winter coat partially shed, his outline was clearly visible. His ribs and spine had fat cover and were not prominent. Neck and hindquarters showed moderate fat cover. As a stallion he had some fatty crest on his neck, a male sexual characteristic indicated by the arrow.

Figure A5.2F: 4-year-old mare, condition score 2.5. Photographed in spring after wintering on hill ground, shedding her winter coat. Ribs and spine had fat cover and were not prominent. Her neck and hindquarters showed moderate fat cover.

Figure A5.2G: 8-year-old mare, condition score 3. Photographed on hill ground in early summer in summer coat, her outline was clearly visible with no winter coat to obscure it. Ribs and spine were well covered in fat deposits. Hindquarters and neck were rounded. Her belly was large and drooping due to pregnancy.

Figure A5.2H: Yearling filly, condition score 3. Photographed in early summer on the hill ground. Yearlings' necks and hind quarters are less developed than in older ponies. Ribs were covered and her hindquarters were rounded. Figure 2I shows the same pony, a year later, with the same body condition score but a different outline due to increased maturity.

Figure A5.2I: 2-year-old filly: condition score 3. The same pony as in figure 2H, the same body condition score, but with a more mature outline. Photographed in spring, on hill ground. Her ribs were covered with fat and her hindquarters were rounded. Her neck showed a more developed crest associated with increased maturity, indicated by the arrow.

Figure A5.2J: 10-year-old mare, condition score 3. Photographed in summer coat on hill ground. Ribs were not visible and covered with fat, back and spine were rounded and well covered. Her neck and hindquarters were rounded outline although her conformation gives her sloping hindquarters.

Figure A5.2K: 2-year-old filly, condition score 3. She was photographed in spring on hill ground in winter coat. An assessment of condition in winter coat requires careful observation as the coat can alter the outline appearance. Her ribs were well covered, her back was rounded, and her spine was well covered in fat. Her neck and hindquarters were covered with fat and had a rounded outline.

Figure A5.2L: 7-year-old mare, condition score 3. Photographed in spring on hill ground in winter coat, showing a different outline to the immature pony in figure 2K but with the same condition score. Ribs, back and spine were covered with fat. Neck was rounded and her hindquarters were not sunken, although her conformation gives them a steep and drooping outline.

Figure A5.2M: 8-year-old stallion, condition score 3. Photographed in spring on hill ground some winter coat. He had a different outline to the immature pony in figure 2K and the mare in figure 2L but the same condition score.

Figure A5.2N: 4-year-old mare, condition score 3.5. Photographed in summer on hill ground in summer coat. Showing a well-rounded outline, ribs not visible, flat back and covered spine due to fat deposits. The same pony showing the same condition score but aged 6, with a more mature outline, can be seen in figure 2P.

Figure A5.2O: 6-year-old mare, condition score 3.5. Photographed in summer on hill ground in summer coat. This pony had a well-rounded outline, her back, ribs and spine well covered by fat deposits.

Figure A5.2P: Colt foal, condition score 4. An un-weaned colt foal photographed in summer on hill ground. His dense foal coat obscured his outline. He had a well-covered neck and well-rounded body. His upright mane made his neck appear cresty, the blue line indicating the outline of the neck below the mane.

Figure A5.2Q: 11-year-old mare, condition score 4. Photographed in summer on hill ground. She had a flat-back, well rounded outline and obvious round belly due to fat deposits. She was not in-foal.

Figure A5.2R: 7-year-old stallion, condition score 3.5. Photographed in winter on hill ground. Heavy winter coat can alter the appearance of an outline; however, he showed notable belly fat deposits. Figure 2S shows the same pony a year later.

Figure A5.2S: 8-year-old stallion, condition score 4. Photographed in winter on hill ground. He had been moved the previous spring onto better quality hill ground. In this photograph, one year later than in figure 2R, he had greater fat deposits on his neck and hindquarters giving him a higher condition score. Figure 2T shows him in the later in the same year.

Figure A5.2T: 8-year-old stallion, condition score 4.5. This is the same stallion as in figures 2R and 2S. Photographed in early summer on hill ground he has shed his winter coat. An arrow indicates his increased belly fat. The same stallion, but with increased condition in late summer can be seen in figure 2W.

Figure A5.2U: 8-year-old mare, condition score 4.5 Photographed in summer on hill ground. A very round outline, with fat deposits along the back such that her that spine was recessed in fat. This can be seen by the channel along her spine visible on her hindquarters.

Figure A5.2V: 8-year-old mare, condition score 4.5. Photographed in summer on hill ground. Her pregnancy changes her outline, her belly is rounded, lopsided and drooping. She has a very heavy neck for a female, with fat deposits on her crest indicated by an arrow, and her spine and tailhead sunken in fat.

Figure A5.2W: 8-year-old stallion, condition score 5. This is the same stallion as in figures 2R, 2S and 2T showing him with condition scores 3.5, 4 and 4.5. Photographed later in the summer he had gained more condition. An obese pony with very a heavy neck and large belly with excess belly fat indicated by the arrow. For welfare reasons he was removed to a different location to reduce his weight after the photograph.

Appendix 6

McMaster Quantitative Faecal Egg Count (FEG)

To assess whether body condition scores were related to parasite loads, nematode burdens were assessed based on faecal egg counts (FEG). This method provides an estimate of worm burden by determining the number of nematode eggs per gram of faeces. A 2-cell McMaster counting slide was used. Eggs were floated free of other debris, then the number of eggs present in the two, lined, 1cm^2 squares of the McMaster slide were calculated, to give an FEG for each individual sample.

While wearing protective gloves, fresh faeces from identified ponies were collected and processed within four hours of collection. Two grams of faeces were added to 60 ml of sodium nitrate specific gravity 1.200 (+/- 0.005) in a beaker, which was then swirled vigorously to homogenise the solution. The solution was then sieved to remove solid matter and samples of the fluid were pipetted into both chambers of the McMaster slide. After waiting <3 minutes so that eggs present in the solution could float to the surface of the suspension, the slide was examined under 10X magnification, and any eggs observed within the gridded areas of both chambers of the slide were counted. The total number of eggs was then multiplied by 100 as per the protocol, to give a calculation of the number of eggs present/g of faecal material (FEG). The FEG scores for each individual were recorded and used as an explanatory variable in the analysis of factors affecting body condition scores.

Protocol for calculation of parasite load using the McMaster floatation technique:

McMaster Quantitative Faecal Egg Count (FEG)

Procedure:

1. Wearing protective gloves, collect a sample of fresh faeces from an identified individual pony.
2. Samples should be processed as soon as possible, always >4 hours after collection.

To prepare sample for egg count:

3. Weigh 2 grams of faeces.
4. Add the 2 grams faecal material to 60 ml of sodium nitrate specific gravity 1.200 (+/- 0.005) in a beaker. Stir or swirl the contents vigorously to break down clumps and homogenize the faecal sample.
5. Pour the contents through a sieve into another beaker. Use a spatula to squeeze as much fluid as possible from the material left in the sieve.
6. While swirling vigorously to maintain the material in suspension, pipette a sample of the mixture and transfer it to one chamber of the McMaster slide.
7. Repeat the procedure to fill the adjacent chamber.
8. Wait 1-3 minutes to allow eggs to float to the top and debris to fall to the bottom of the chamber.
9. Under 10X power, count the eggs that fall within the gridded area of both sides of the chamber. (Do not count any eggs that fall outside the grid.)
10. Multiply the total number of eggs in the 2 chambers by 100; this is the eggs per gram of faeces. (FEG)
11. McMaster slide can be washed with detergent between uses.

Appendix 7

Table A7.1 Details of the 55 WGS samples collected from Exmoor ponies. Sire and dam lines as recorded in the EPS Stud Book.

Sequence ID	Pony number Current herd	Sex	Status	WGS Sequencing date	Sire line Founder herd	Dam line Founder herd
E_102004	102/004	M	Alive	2022	1010	900035
E_107013	107/013	M	Dead	2022	12002	900051
E_21084	021/084	M	Alive	2022	48010	1035
E_21149	021/149	F	Alive	2022	1009	12011
E_23279	023/279	M	Alive	2022	48010	23008
E_23416	023/416	M	Alive	2022	48010	1035
E_23434	023/434	F	Alive	2022	48010	23001
E_320005	320/005	F	Alive	2022	1010	12011
E_32023	036/023	M	Alive	2022	12002	12011
E_44009	044/009	F	Alive	2022	1009	44002
E_458028	458/028	F	Alive	2022	1010	900049
E_479023	479/023	F	Alive	2022	12002	12011
E_49031	049/031	M	Dead	2022	1009	12002
E_49057	049/057	F	Dead	2022	1009	12002
E_49121	049/121	F	Alive	2022	1009	76001
E_512001	512/001	F	Alive	2022	1010	23008
E_519005	519/005	M	Alive	2022	1010	900005
E_78170	078/170	F	Alive	2022	1009	1028
E_900234	000/243	F	Alive	2022	1010	900002
E_900585	000/585	F	Alive	2022	1010	27001
E_900588	000/588	F	Alive	2022	1010	900051
E_900600	000/600	F	Alive	2022	1010	900041
E_900694	000/694	F	Alive	2022	1009	76003
E_900717	000/717	F	Alive	2022	1009	900052
E_900741	000/741	F	Alive	2022	1010	900054
s12157	012/257	F	Alive	2021	1010	1020
s2010	002/010	M	Dead	2021	1009	12002
s2012	002/012	M	Alive	2021	48010	76001
s21098	021/098	F	Alive	2021	1009	900050
s21131	021/131	F	Alive	2021	1009	900051
s21134	021/134	F	Alive	2021	1009	1020
s23203	023/203	F	Alive	2021	48010	23010
s235016	235/016	F	Alive	2021	1009	1031
s237009	232/009	F	Dead	2021	48010	900003
s276021	276/021	F	Alive	2021	12002	900051
s335004	335/004	F	Alive	2021	12002	12011

s479013	479/013	M	Dead	2021	1010	12011
s479021	479/021	F	Alive	2021	1010	12011
s479022	479/022	F	Dead	2021	1010	1031
s49011	049/011	F	Dead	2021	1010	12002
s49022	049/022	F	Dead	2021	1009	12002
s49052	049/052	F	Dead	2021	1009	12002
s49097	049/097	M	Dead	2021	1009	76001
s49124	049/124	M	Alive	2021	1009	76001
s49125	049/125	F	Alive	2021	1009	1020
s49127	049/127	F	Alive	2021	1009	12002
s49145	049/145	F	Dead	2021	1009	76001
s49147	049/147	F	Alive	2021	1009	76001
s49149	049/149	F	Alive	2021	1009	76001
s78150	078/150	F	Alive	2021	1009	1020
S21029	021/029	F	Alive	2020	12002	1020
S21092	021/092	F	Alive	2020	1009	1030
S21109	021/109	F	Alive	2020	1009	1030
S21140	021/140	F	Alive	2020	1009	1035
S276023	276/023	M	Alive	2020	12002	900051
S49052	049/052	F	Dead	2020	1009	12002

Appendix 8

DNA extractions

DNA was extracted using Qiagen DNeasy kits (DNeasy blood and tissue kit, QIAGEN, Valencia, CA.) using the protocols supplied using the following steps:

1. 1cm of hair cut from the end of 10-20 hairs with scissors into a sterile petri dish.
2. Instruments UV-light treated between samples.
3. Hair transferred into a 1.5ul Eppendorf tube containing 180ul ATL (lysis) buffer, (to digest the cells and release DNA), and 20ul ProteinaseK solution, (to denature released proteins which may degrade the DNA).
4. Overnight 56 ^{0}C incubation with agitation.
5. Add 4ul RNase (100mg/ul) and incubate at 37^{0} C for 30 minutes (removal of RNA).
6. Centrifuge
7. Pipette supernatant into a fresh 1.5ml Eppendorf tube
8. Add 200ul of AL (lysis) buffer (to complete cell lysis)
9. Incubate at 70^{0} C for 10 minutes

DNA precipitation:

Add 200ul of 100% ethanol

DNA purification:

1. Pipette solution into a DNeasy column collection tube
2. Centrifuge at 6000g for 1 minute,
3. Discard flow through and collection tube
4. Place DNeasy column into a new collection tube
5. Add 500ul AW1 (wash buffer)
6. Centrifuge at 6000g for1 minute
7. Discard flow through and collection tube
8. Place DNeasy column in a new collection tube
9. Add 500ul AW2 buffer
10. Centrifuged for3 minutes at 20,000g
11. Discard flow through
12. Place column in the collection tube
13. Centrifuge at 20,000g for 1 minute (to dry the membrane).

DNA elution:

1. Place the DNeasy column in a new 1.5ml Eppendorf tube
2. Add 42ul of AE elution buffer
3. Leave on the membrane for 1 minute
4. Centrifuge for 1 minute at 6000g
5. Add 42ul of AE elution buffer
6. Leave for 1 minute
7. Centrifuge for 1 minute at 6000g
8. To increase DNA yield, repeat with 22ul of AE buffer

Appendix 9

Table A9.1: mtDNA haplotypes and their associated founder matrilines as shown in the genetic load in relation to inbreeding figures

mtDNA haplotype	**Haplotype Sub-division**	**Associated matrilines**
1		12011, 27001
2		76001
4		23008, 23010
5		1028, 1035, 23001
6	6.1	12002
	6.2	12011
7	7.1	1020, 1030, 1031, 1035, 48029
	7.2	900002, 900003, 900005, 900041, 900049, 900050, 900051, 900052, 900054

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