A single introduction of wild rabbits triggered the biological invasion of Australia

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Abstract

Biological invasions are a major cause of environmental and economic disruption. While ecological factors are key determinants of their success, the role of genetics has been more challenging to demonstrate. The colonisation of Australia by the European rabbit is one of the most iconic and devastating biological invasions in recorded history. Here, we show that despite numerous introductions over a 70-year period, this invasion was triggered by a single release of a few animals that spread thousands of kilometres across the continent. We found genetic support for historical accounts that these were English rabbits imported in 1859 by a settler named Thomas Austin and traced the origin of the invasive population back to his birthplace in England. We also find evidence of additional introductions that established local populations but have not spread geographically. Combining genomic and historical data we show that, contrary to the earlier introductions which consisted mostly of domestic animals, the invasive rabbits had wild ancestry. In New Zealand and Tasmania, rabbits also became a pest several decades after being introduced. We argue that the common denominator of these invasions was the arrival of a new genotype that was better adapted to the natural environment. These findings demonstrate how the genetic composition of invasive individuals can determine the success of an introduction and provide a mechanism by which multiple introductions can be required for a biological invasion.

Significance Statement

Biological invasions are a major global threat and understanding what promotes their success is crucial to developing mitigating policies. While properties of the invasive species or environment have been associated with the success of biological invasions, the role of genetics has been more challenging to demonstrate. Combining genomic and historical data, we provide this link by showing that one of the most iconic biological invasions was triggered by a single introduction of rabbits into Australia, which were likely better adapted to the natural environment due to their wild ancestry. Before the arrival of this lineage, numerous earlier introductions failed to spread, suggesting that the genetic composition of the introduced individuals played a crucial role in determining the invasion’s success.

Introduction

When organisms spread beyond their native range, they often either establish localised populations or do not survive. However, occasionally exotic species proliferate and outcompete well-adapted native species. These events, known as biological invasions, are a major cause of environmental (1) and economic disruption, with an estimated global cost of US$1.288 trillion over the last 50 years (2). In an increasingly cosmopolitan world where human activity and climate change are moving species beyond their native range at ever-increasing rates, the risk of biological invasions has never been higher. Due to this devastating and often irreversible impact, the reason why some introductions lead to biological invasions, but others do not, has attracted considerable attention (3, 4).

Ecological factors are critical for biological invasions, with the properties of certain species making them successful invaders and the properties of some environments making them vulnerable to invasion (5). The genetics of invasive populations has also been shown to play an important role in the outcome of these processes (6). More recently, it has become apparent that propagule pressure—the number of introductions and the number of individuals introduced—play a key role by helping overcome stochastic processes that can lead to population extinction (3, 4). However, it has also been argued that high propagule pressure may allow established but localised populations to become invasive by altering the genetic makeup of the introduced population (4). One mechanism by which this can occur is introducing greater genetic variation, which may reduce inbreeding depression or provide the genetic variation that natural selection can act on to adapt the population to the new environment (3, 4). Alternatively, high propagule pressure can also increase the probability that an invasive adaptive genotype will be introduced (4, 7).

To understand the role of genetic factors in biological invasions, we combined genetic data and historical records to investigate one of the most iconic and thoroughly recorded biological invasions in history, the rabbit colonisation of Australia. For most of its existence, the European rabbit (*Oryctolagus cuniculus*) was restricted to the Iberian Peninsula and the South of France (8, 9). During the Middle Ages, rabbits were extensively translocated by humans and today rabbits are one of the most widespread mammals, with a presence across multiple continents and in

hundreds of islands spread around the globe (10, 11). Despite being a keystone species in the native range (12), rabbits are considered pests in most introduced locations, responsible for damage to agriculture, habitat degradation, and endangerment of native species (13). This invasive potential has been recorded throughout human history and goes as far back as 30BC when Strabo (Geographica, III, v) describes a rabbit infestation in the Balearic Islands so large that inhabitants had to request help from the Roman Emperor. Moving forward 1500 years, and the Portuguese historian João de Barros (1496-1570) describes a 15th-century settlement on the island of Porto Santo, Madeira, that had to be abandoned due to a rabbit infestation that originated from a single pregnant doe (14). Of all the biological invasions by rabbits, the impact on Australia was the greatest, leading farmers to abandon properties overrun by rabbits and disrupting the entire agricultural sector (15, 16). Despite the efforts to control the population numbers, rabbits are still one of the major invasive species in Australia where they impact native flora and fauna (17) and are responsible for an estimated annual cost to the agriculture industry alone of $200 million, 22 times the value for feral pigs (18).

It is common to observe that there is a time lag between species being introduced and becoming invasive (19), and this poorly understood phenomenon is clearly illustrated by Australian rabbits. Rabbits were first introduced to mainland Australia when five domestic rabbits were brought to Sydney on the First Fleet in 1788, as stated in the account of the settlement livestock (20). Decades after, rabbits were commonly bred in houses around Sydney (21). In years that followed the first importation, rabbit translocations were frequent and rabbit warrens were reported all over the country (22). By 1870, rabbits were widely kept in the major settlements along the coast (22). These populations were often described as having a domestic origin, which is likely since wild rabbits were not easy to get hold of and were less suitable for transportation, breeding and management compared to their domestic counterparts (20). The domestic origin of these populations is supported by reports of traits which are normally absent in wild rabbits, such as tameness, fancy coat colours and floppy ears (20–22). Despite the presence of rabbits across Australia, the vast majority of the populations either failed to establish in the wild or did not spread beyond their local range (21, 22). However, in the second half of the 19th-century rabbit populations increased dramatically and spread across the country

(21). At a rate of 100 kilometres per year, it took rabbits 50 years to cover an area 13 times the size of their native range in the Iberian Peninsula, making this the fastest colonisation rate for an introduced mammal ever recorded (21). By the beginning of the 20th century, rabbits were a conspicuous feature of the Australian landscape, in what has been described as a 'grey blanket' covering the land (15).

The population growth observed in mainland Australia in the late 19th century was replicated in New Zealand and Tasmania. In both locations, rabbits were commonly traded during the early 1800s, and while local populations existed, they did not spread and became invasive (11, 22). These early introductions were also likely from domestic stock, with some records explicitly mentioning the introduction of domestic rabbits (15), and even specific breeds such as lop-eared rabbits in New Zealand in 1856 (20, 23). However, in the 1860s, rabbit numbers started to increase at a rapid rate, ultimately becoming a nuisance that demanded pest control in both locations (11, 22).

In the historical literature, the transition from rabbits being a localised species to becoming invasive is frequently attributed to a single introduction. Thomas Austin, an English settler aiming to establish a rabbit population for hunting in his estate in mainland Australia, requested that his family in England send some rabbits (20, 24). On the 6th of October of 1859, Thomas' brother James sent on board the ship *Lightning* a consignment of domestic and wild rabbits caught around the family property in Baltonsborough, South East England (20, 24). On the Christmas day of that same year, the consignment arrived in Melbourne with 24 rabbits on board (25, 26). These rabbits were taken to the property of Thomas Austin in Barwon Park, near Geelong in Victoria. Within three years, the 1862 Chronicle stated how 'Austin rabbits' numbered in thousands (20) and in 1865, Austin himself reports to the *Geelong Advertiser* how he killed 20,000 rabbits at his estate, as a statement to the "extraordinary fecundity of the English rabbit". By 1906, rabbits had covered thousands of kilometres reaching the West Coast, and historical reports have classically claimed that they have expanded from Barwon Park. Despite this popular belief, previous studies have failed to find a genetic pattern in Australian rabbit populations consistent with this expansion (27) and a recent genome-wide study disputed the single-origin hypothesis, instead arguing that invasive rabbits arose from several independent introductions (28).

Why did rabbits change from being a localised and innocuous species to becoming invasive? Anthropogenic changes to the environment, such as the development of large pastoral areas and predator populations being controlled by pastoralists, were beneficial for rabbits and might have made mainland Australia progressively more vulnerable to an invasion (21). However, the observed time lag between rabbits establishing populations and becoming invasive was also replicated in other locations, such as New Zealand and Tasmania, suggesting that other factors were at play. These parallel changes in rabbit population dynamics across three locations with such different environmental conditions suggests that non-environmental factors might have played a crucial role in the success of this biological invasion. One potential explanation is the introduction of novel rabbit genotypes that were better adapted to the natural environment, and the wild genetic ancestry of Thomas Austin rabbits might provide the mechanism by which this happened. This is plausible as Austin's release is the only historical record explicitly stating the release of wild rabbits into mainland Australia that we are aware of (22), and Austin rabbits were then introduced to New Zealand during the 1860s, when rabbits started to become a pest (15, 20).

To investigate the causes of the biological invasion, we analysed genetic data from rabbits collected across mainland Australia, Tasmania and New Zealand, together with populations that might have contributed to the Australasian gene pool (Figure 1). This allowed us to test whether invasive rabbits in Australia arose from a single introduction or multiple introductions. This is important, as if the trigger for the invasion was environmental change, then multiple local populations would likely expand. However, if the trigger was the arrival of a specific invasive genotype, then rabbits from across the country would be derived from that introduction. Second, we test whether invasive rabbits have wild ancestry, which provides an explanation of why they were better adapted to local conditions than early introductions. Finally, we link our data to the historical record by investigating whether the release by Thomas Austin gave rise to the invasive genotype of rabbits.

Results

We have analysed whole-exome sequences of 187 individuals belonging to i) Australasian populations of mainland Australia (n=62), Tasmania (n=2) and New Zealand (n=5), ii) wild rabbit populations from France (n=55) and Britain (n=55); and iii) domestic rabbits belonging to eight different rabbit breeds (n=8) (Table S1). The average coverage across samples was 30.5x. The capture targeted a total of 32.10 Mb, which corresponds to 1.17% of the genome. The total number of variants after filtering was 1,987,606.

Sequential colonisations reduced the genetic diversity of rabbit populations

Australian rabbits are thought to be the result of a sequential colonisation process that was initiated when rabbits were introduced from continental Europe into Britain, and from there into Australia. The population bottlenecks that accompanied these introductions have resulted in a 10.6% reduction of genetic diversity from continental Europe (France) to Britain and 12.3% from Britain to mainland Australia (Figure 2A, Table S2). This modest reduction in genetic diversity has been reported before (29), and is expected if the population bottleneck associated with colonisation was followed by a rapid population expansion.

In addition to a decrease in nucleotide diversity, recent population bottlenecks lead to a preferential loss of rare genetic variants (30). To examine this pattern, we used sequences from a hare to classify alleles as ancestral or derived, and plotted the unfolded allele frequency spectrum. In support of sequential population bottlenecks, the highest number of low-frequency alleles was in France followed by Britain and then mainland Australia (Figure 2B). This is reflected in Tajima's *D* statistic —a summary of the allele frequency spectrum— which becomes progressively larger from France to Britain and then Australia (Table S2).

Invasive rabbits arose from a single introduction into mainland Australia

While many accounts attribute the origin of Australian rabbits to a single introduction in 1859, some genetic analyses and historical records suggest that the current rabbit population is the result of multiple introductions and translocations (22, 28). To

resolve this, we looked at the patterns of genetic structure in populations across mainland Australia (Figure 3A). Our results showed a high level of genetic similarity across regions, with the exception of five rabbits from two locations. This is shown in a principal component analysis (Figure 3B), where mainland Australian rabbits fall into three distinct groups, the largest of which (57 out of 62 individuals) includes rabbits from across the country, covering an area spanning thousands of kilometres. Two smaller clusters were found in a far smaller geographic region – one group of four rabbits was from Sydney, and the other was a single rabbit from Cattai National Park, which lies Northwest of Sydney. To corroborate this result, we used a neighbour-joining tree to cluster rabbits by their genetic similarity (Figure S1). Again, the rabbits from Sydney and Cattai clustered independently from the main group of rabbits from elsewhere in Australia.

Wild British and Domestic Rabbits were introduced into mainland Australia

Historical accounts of the origin of Australian rabbits vary, with most records referring to initial introductions of domestic rabbits and others mentioning a later introduction of wild British rabbits. We investigated the source of these introductions with an *Admixture* analysis, which assumes rabbit genomes are a mixture of discrete ancestral populations (Figure S2) (31). This analysis corroborated our PCA and neighbour-joining tree, revealing different ancestries of the three distinct genetic groups in mainland Australia (Figure 3C, K=3). Most rabbits from across the continent have a distinct ancestry fraction of their own, likely reflecting a population bottleneck that has made it genetically distinct from the source population (32). The Sydney rabbits appear to be predominantly derived from domestic rabbits, in line with historical records of five domestic rabbits that were carried to Sydney on the First Fleet in 1788 (20). The largest ancestry fraction in the Cattai rabbit genome is shared with British rabbits, suggesting that there was a separate introduction from Britain into this region. These patterns of ancestry are also supported by our earlier analyses – in both the PCA and the neighbour-joining tree, the Sydney population is most similar to domestic rabbits while the Cattai rabbit falls within the British population (Figures 3B and S1). The domestic ancestry of Sydney rabbits is further supported by their mitochondrial genome sequences (mtDNA). All the Sydney

rabbits shared an identical mtDNA haplotype, and this is closely related to the haplotype found in most domestic rabbits (Figure 4A and S3).

To investigate the relative contributions of domestic and wild British rabbits to the mainland Australian population, we calculated outgroup *f3* statistics (33). Using France as the outgroup, this statistic allows us to use correlations in allele frequencies to examine the extent to which pairs of populations share genetic drift, and therefore a common ancestry. This revealed that the Cattai rabbit had the greatest wild British ancestry and Sydney the least (Figure 3D Top). Rabbits from other regions of Australia were intermediate. This pattern was reversed when considering domestic ancestry (Figure 3D Bottom). Sydney has the greatest domestic ancestry, Cattai the least, and the rest of the country was intermediate. Therefore, these results demonstrate that the main genotype of invasive Australian rabbits has a mixed domestic and wild ancestry.

To reconstruct the historical relationships among our populations, we divided mainland Australia into subpopulations and reconstructed their relationships using the *TreeMix* method (Figure 3E), which uses population allele frequencies to construct a tree of populations. This confirmed that the Cattai rabbit is genetically distinct and is more closely related to British rabbits than those from the rest of Australia, consistent with it being derived from a separate introduction of British rabbits. The Sydney population is most closely related to domestic rabbits. Populations across the rest of the mainland are closely related and have an intermediate position on the tree. Despite rabbit domestication occurring in France (34, 35), on our tree domestic rabbits and French rabbits do not fall into the same clade. This might reflect our failure to sample the French population that gave rise to domestic rabbits, or that mixing between populations obscures some population relationships as they cannot be represented by a bifurcating tree.

Mitochondrial DNA suggests the number of female rabbits introduced to Australia was small

To investigate the evolutionary history of the female lineage of Australian rabbits, we reconstructed the genealogy of mitochondrial genome sequences that cover the colonisation route from Continental Europe to Australia. We included the population

of origin as a discrete trait during the reconstruction of the tree, allowing us to reconstruct past migrations of female rabbits. By inferring the ancestral location of mitochondrial lineages, it is apparent that mainland Australian rabbits fall into a small number of clusters on the tree, indicating that they are derived from a small number of female rabbits introduced from elsewhere (Figure 4A). This is consistent with historical records suggesting that the Barwon Park release may have been derived from as few as 13 animals (see Discussion). To quantify the number of introduced female rabbits that gave rise to the mitochondrial genomes in our sample, we counted transitions between countries while accounting for uncertainty in the tree topology (Markov jumps; (36)). From this, we estimate that the mainland Australian rabbits in our dataset trace their maternal ancestry back to five females that were introduced from Europe (Figure 4B; 95% credible interval: 3-5 rabbits).

A single introduction rapidly expanded to colonise most of Australia

Historical records report an extremely rapid population expansion of rabbits across mainland Australia from Victoria, where Thomas Austin property, Barwon Park, is located (Figure 1). As individuals move further away from the population's source and new regions are colonised, allele frequencies change due to genetic drift. Consistent with this, we found a correlation between genetic and geographic distance between pairs of individuals sampled from across the country ($r = 0.361$; Mantel Test: $P < 0.001$, Figure 5A, red points, excluding Sydney and Cattai). However, the genetic distance between Sydney/Cattai rabbits and the rest of mainland Australia is consistently greater than expected given the geographic distance between samples (Figure 5A, grey and white points). This supports the hypothesis that most mainland Australian rabbits arose from a single introduction that expanded across the continent, but rabbits in Cattai and Sydney have separate origins.

A principal components analysis of mainland Australian rabbits, where samples are coloured according to the distance to Barwon Park, further describes this pattern of range expansion (Figure 5B; analysis excludes Sydney and Cattai). The first principal component reflects the initial northward expansion of the population, while the second principal component separates individuals from Western Australia and

Queensland on an East-West axis. This likely reflects the routes taken to colonise these more distant regions after the initial expansion to the North of Barwon Park.

As populations expand and new areas are colonised, repeated founder effects can lead to a loss of genetic diversity (37). Therefore, we tested whether the genetic diversity declined with increasing distance from the point of introduction at Barwon Park (Victoria), by calculating the genetic diversity of rabbit populations across mainland Australia. Since our sampling is not uniform, we focused on four distant locations (Victoria/NSW, South Australia, Queensland, and Western Australia). The closest individual to Barwon Park for each of these locations was at a distance of 72 km, 979 km, 1323 km and 2521 km, respectively. We found a decrease in genetic diversity as populations get more distant to Barwon Park, with Victoria/NSW being the most diverse population and Western Australia the least diverse (Figure 5C).

Alongside the decrease in genetic diversity, a process known as allele surfing can drive rare alleles to high frequencies during geographical expansions (38, 39). This happens when a new mutation or rare allele finds itself at the front of the wave of expansion where it benefits from rapid population growth. The rabbit colonisation of mainland Australia, since it likely originates from a single introduction that rapidly expanded across a large geographical area, represents an ideal framework to empirically test this theoretical prediction in a natural setting. To select variants that were rare or absent in the rabbits initially introduced into mainland Australia, we identified alleles that were absent from our samples of British and domestic rabbits (the two populations that gave origin to mainland Australian rabbits). As predicted by the allele surfing model, these initially rare alleles were more likely to have increased in frequency the further you travel from the release site in Barwon Park, Victoria (Figure 5D).

Mainland Australia rabbits came from the South-West of England

The historical records describe that the British wild rabbits imported by Austin were captured around his family's property in Baltonsborough, Somerset (see Introduction). To test whether this is correct, we looked at the correlation in allele frequencies between different areas of Britain and mainland Australia. We grouped the British samples by the county in which they were collected, then calculated *f*3

statistics between these populations and mainland Australia (excluding Cattai/Sydney), while using France as an outgroup. This revealed that Hampshire, Dorset and Glamorgan (Figure 6A, red circles) were the three locations with the greatest genetic similarity to mainland Australian rabbits. Strikingly, these populations are all in the South-West of Britain, near Baltonsborough (Figure 6A, Figure S4).

As an alternative approach to investigate the source of mainland Australian rabbits, we examined rare variants. These are expected to be highly differentiated between closely related populations, making them informative about recent demographic events (40, 41). For each British rabbit, we took variants that were not found in any other British rabbit we sampled (i.e. they were singletons) and asked what proportion of these were found in our mainland Australian samples. To avoid the confounding effects caused by the mixed domestic/British ancestry of Austin's rabbits, we excluded variants that were present in the domestic population. We found that rare variants were more frequently shared between rabbits in South West England and mainland Australia, again supporting the hypothesis that the source of the invasive population was Baltonsborough (Figure 6B; Pearson's correlation between proportion shared singletons and the distance to Baltonsborough: r=0.611, p < 0.001).

Tasmanian populations are mixed domestic and mainland Australian rabbits

In order to investigate the origin of Tasmanian rabbits, we sequenced two individuals collected from geographically distant locations. These rabbits were the least genetically diverse in our dataset (Table S2). Multiple lines of evidence suggest that domestic rabbits contributed majorly to the Tasmanian genetic pool. First, in our population tree, PCA and neighbour-joining tree Tasmania is consistently more closely related to Sydney and domestic populations (Figure 3E). Second, in the *Admixture* analysis, the largest ancestry component is shared with domestic rabbits (Figure 3C). Finally, the *f*3 statistic shows a substantial contribution of domestic ancestry, only superseded by Sydney rabbits (Figure 3D bottom). Together these results suggest that Tasmanian rabbits, like Sydney, are derived in large part from domestic stock.

Historical records report that Tasmanian rabbit populations increased in size shortly after the Barwon Park release on mainland Australia, suggesting that these rabbits may have been released in Tasmania (see Introduction). Consistent with this, the *Admixture* analysis shows that the Tasmanian rabbits are mixed between domestic and mainland Australian populations (Figure 3C). Furthermore, the Tasmanian rabbits are intermediate between domestic and mainland Australian rabbits on the PCA (Figure 3B), and the analysis of mitochondrial DNA found evidence of female rabbits being introduced from the mainland into Tasmania (Figure 4B). There is no significant support in our data for direct introductions of rabbits from Britain into Tasmania, with the PCA, *Admixture* analysis, and *f*3 statistics all showing no evidence of direct British ancestry (Figures 3B, 3C and 3D).

New Zealand rabbits have mixed wild British, domestic and mainland Australian ancestry

We sequenced five rabbits from New Zealand, sampled from the two major islands, the North (n=2) and South Islands (n=3). Our results clearly suggest that domestic rabbits are an important component of the ancestry of New Zealand populations. The *f*3 statistic indicates similar levels of domestic ancestry in New Zealand and mainland Australian populations (Figure 3D, bottom), while the *Admixture* analysis shows a substantial domestic ancestry (Figure 3C). There is also evidence of direct introductions from Britain into New Zealand, with both the *f*3 statistic and Admixture analysis showing more British ancestry than is the case for Sydney or Tasmania (Figure 3C and 2D, top). In fact, the level of British ancestry in the *Admixture* plot for three New Zealand samples is the highest in the dataset with the exception of Cattai. This is also supported by our analysis of mitochondrial genomes, where there is support for direct introductions from Europe (Figure 4A). Furthermore, the *Admixture* analysis (Figure S2 - *K*=7) and *f*3 statistics (Figure S4 B and D) both suggest that New Zealand rabbits are more related to populations in eastern England, unlike Austin rabbits which originate from southwest England.

The degree of British ancestry varies among our samples, resulting in marked genetic structure within New Zealand (Figures 3B, 3C and S1). To investigate this pattern further we split the New Zealand population into the two groups seen on the

PCA and reconstructed the population relationships using *Treemix*. This confirmed that some New Zealand rabbits are closely related to domestic rabbits, but others are more related to British rabbits (Figure S5). These differences are not associated with whether the rabbit comes from the North or South Island, suggesting the existence of local populations with independent origins. Together these results indicate that there were direct introductions from Britain into New Zealand, but the extent of British ancestry varies between samples. There is also evidence of introductions of mainland Australian rabbits into New Zealand, with the smallest ancestry component in our *Admixture* analysis being shared with mainland Australia (Figure 3C).

Discussion

A major question in ecology is why do some introductions become biological invasions, but others do not? However, the multitude of concurrent factors that are at play during the incipient stages of a biological invasion, and the lack of detailed records on the origin, number, and timing of each introduction, makes dissecting this process challenging. The rabbit colonisation of Australia was accompanied by detailed historical literature on the events and people involved, providing a unique opportunity to combine genetics and history to understand one of the most iconic biological invasions of all time and examine the factors that led to its success (Figure 1). The historical literature on rabbits in Australia records a common pattern in biological invasions—initially, there were numerous introductions that established small local populations, but after a time lag the population size dramatically increased and rabbits became invasive. A key question is, therefore, what changed to cause rabbits to become invasive?

While biological invasions are often attributed to properties of the invasive species or the environment, there is growing evidence for the importance of propagule pressure—the number of introductions and the number of individuals introduced (4). In the case of rabbits, there are historical records of over 90 importations into mainland Australia before 1859, when Thomas Austin released wild English rabbits at Barwon Park. Of these 90, at least 30% were reported as releases into the wild (22). Whether from the original 1788 introduction of domestic rabbits brought to Sydney in the first fleet or the subsequent releases, we found support for the domestic ancestry of modern Sydney rabbits in multiple analyses.

Despite evidence of widespread rabbit introductions, it took over seven decades from the arrival of rabbits in Sydney for the biological invasion to occur. The natural barrier imposed by the densely forested Great Dividing Range may have prevented the westward expansion of Sydney rabbits, but this would not have affected populations established elsewhere. More likely, early rabbit introductions may not have become invasive because of environmental factors that later changed with anthropogenic pressure to make the landscape vulnerable to invasion. In particular, the expansion of the pastoral industry would provide a continuous source of food for a growing rabbit population (16). Furthermore, pastoralists suppressed predator

populations, and there is extensive evidence that predators control the rabbit populations in mainland Australia (22). If environmental change was the sole trigger for the invasion, then one would predict that multiple local populations would have expanded their range. Instead, our results provide clear genetic evidence that invasive mainland Australian rabbits result from the single introduction, suggesting that these rabbits were genetically more prone to invasion than previous releases. This supports the historical record that suggests that the invasive genotype was released in 1859 by Thomas Austin on his property at Barwon Park in Victoria.

The dynamics of the rabbit invasion of mainland Australia, due to its speed, magnitude, geographical range and known origin, provide an ideal dataset to test population genetics theory. As rabbits move away from Barwon Park, genetic diversity declines, consistent with recurrent founder events at the front of the wave of expansion. Alongside the loss of variation, rare alleles that occur in the rapidly growing populations at the front of the range expansion can rise up in frequency due to drift, a process known as allele surfing (38, 39). Despite the extensive literature on allele surfing theory, few studies have demonstrated it empirically (42). We found that alleles that are rare or absent in the source population are more likely to be common in the populations further away from the origin of the invasion in Victoria.

When combining our results with the historical record, it becomes clear that rabbit introductions were common across Australia after rabbits first arrived in 1788, sometimes establishing local populations. In addition to Sydney, we found evidence of another introduction of British rabbits that did not become invasive. This was based on a single sample from Cattai National Park, 50km from Sydney. Throughout our analysis, this individual consistently appeared more closely related to wild British rabbits than mainland Australian rabbits. The high divergence of Cattai rabbits was also noticed by Phillips *et al.* when comparing mtDNA haplotype frequencies across Australia (43). It is unclear why these rabbits did not become invasive, but it is possible the Cattai release occurred after surrounding regions were colonised by rabbits from Barwon Park.

Our finding of separate introductions into Sydney and Cattai highlights the possibility of other introductions that exist as local populations that we did not sample. Historical records from 1870 mention a second major rabbit spread at Kapunda, South Australia that ranks in importance with the Barwon Park release, and suggest

it merged with the expansion from Barwon Park in 1979 (16, 21). We did not find evidence of an introduction of a different rabbit stock close to South Australia. This could mean that this release likely originated from the same stock of Barwon Park or that this population did not expand into the region we sampled. It is likely that finer sampling would reveal additional populations that have not spread geographically and whose origin is independent of Barwon Park. Nevertheless, our results provide overwhelming evidence that the large majority of mainland Australian rabbits derive from a single introduction by Thomas Austin.

Our findings contrast with a recent genetic study that argued that invasive rabbits arose from multiple introductions into mainland Australia (28). As acknowledged by the authors, they did not sample the ancestral European and domestic populations which were critical for us to discern that invasive rabbits arose from a single introduction. Without this information, the authors based their conclusions on two arguments. First, they find no signal of isolation by distance. However, this may have been obscured by the inclusion of a large sample from the separate introduction into Sydney (and potentially other releases missing from our dataset). Second, the authors interpret substructure within mainland Australia, together with the high number of private alleles in populations such as Melbourne and Sydney, as an indication of independent introductions. While this is the case for Sydney (also supported by our data), these effects can also be explained by the effects of a population expansion on genetic diversity.

A critical question is why the rabbits released at Barwon Park became invasive while numerous other releases of rabbits did not? Our results support the hypothesis that the genetic composition of these rabbits was critical. Austin rabbits were described as wild-caught rabbits from England (21), and our data provide clear support for the wild ancestry of these individuals. Moreover, mainland Australian rabbits are genetically closest to rabbits in Southwest England, where the Austin rabbits were caught. Our results are consistent with the words of Joan Palmer, a Thomas Austin's relative: "(...) When my grandfather, William, was asked by uncle Thomas to send out a consignment of a dozen or so for Barwon Park, he found it quite a difficult assignment as wild rabbits were by no means common round Baltonsborough. It was only with great difficulty that he managed to get six; these were half-grown specimens taken from their nests and tamed. To make up the number he bought

seven grey rabbits that the villagers had kept in hutches, either as pets or to eat. (...)". The invasive Australian rabbits also contain a substantial element of domestic ancestry, which is consistent with Barwon Park rabbits originating from wild and domestic rabbits that bred during the trip. Although our data cannot rule out interbreeding occurring after arrival in Australia, the discrepancy between 13 animals sent from Britain and the 24 that arrived in Australia, suggests that they likely bred before or during the 80 days of the journey, as recounted by Joan Palmer. This small number of animals is also consistent with our mitochondrial analysis that estimates mainland Australian rabbits in our sample to be derived from five introduced females.

The time lag between the first introduction and the biological invasion that was observed in mainland Australia was repeated in Tasmania and New Zealand. It is likely that the introduction of rabbits with wild British ancestry may have triggered these invasions too. In both locations, historical documents record that feral rabbit populations persisted for decades without becoming a serious pest (see Introduction). However, almost simultaneously in both locations, the rabbit numbers exploded in the 1860s following Austin's importation to mainland Australia. There is historical evidence that shows successful liberations of rabbits in New Zealand between 1864 and 1867, which included a batch of rabbits provided by Austin himself (15, 20), and earlier records mention a successful release of rabbits described as wild-type in 1858 (23). Moreover, phenotypic changes suggest a shift of classic domestic traits to wild ones that coincided with rabbits becoming invasive. In Tasmania, James Calder, Surveyor-General of Tasmania, commented in 1869 that the increase in population size coincided with a shift in the colouration to the grey coat colour seen in wild English rabbits (20). Our data support that Tasmania and New Zealand rabbit populations have a substantial component of wild ancestry. In the case of Tasmania, our data shows this came via mainland Australia and in the case of New Zealand it came directly from Britain. Combined, our genetic and historical evidence support that the expansion of the rabbit populations was linked with the introductions of a wild genetic ancestry.

Even when our data shows that there is substantial domestic ancestry in populations such as in Sydney, Tasmania and New Zealand, the arrival of rabbits with wild British ancestry may still have been the trigger for the biological invasion to occur. When an invasive population expands into areas already occupied by small local

558 populations, there can be extensive genetic introgression from the resident
559 population into the invasive population (44). This occurs because when the first
560 invasive immigrants arrive, they mate with resident animals, so alleles from the
561 resident population can become established as the invasive population expands.
562 The result is extensive asymmetric introgression from the original resident population
563 into the invasive population (44).

564 There are many traits that could make feral rabbits poorly adapted to survive in the
565 wild. Domestic animals, including rabbits, differ substantially from their wild
566 counterparts in traits ranging from morphology (*e.g.* coat colour and size) to
567 behaviour (*e.g.* tameness and fear response) (45, 46). This is a well known
568 phenomena in conservation biology, where the hybridisation of feral and wild animals
569 poses a risk to the viability of wild populations by eroding genetic diversity and
570 allowing the introgression of maladaptive alleles (47, 48). In the case of rabbits, feral
571 populations can thrive, but this occurs mostly on islands where predation and
572 competition are often less intense—on islands tameness often evolves without
573 domestication (11, 49, 50). The wild genetic ancestry of populations may also have
574 affected their ability to evolve novel adaptations to the Australian environment. The
575 majority of Australia has an arid or semi-arid climate, and this has led to rabbits
576 evolving changes in body shape that aid thermoregulation (51). It is possible that
577 early feral populations may have lacked the genetic variation required to adapt to
578 these conditions.

579 More than 150 years have passed since Thomas Austin asked his brother to send
580 him some wild rabbits from their family property in England. Unbeknown to him, this
581 request caused a cascade of events that changed forever the landscape of an entire
582 continent and resulted in the greatest pastoral pest of the 20th century. Here, we
583 combined historical records with genetic data, in order to reconstruct the colonisation
584 route of rabbits from the Austin's family property in the south of England to the far
585 end of the rabbit expansion range in Western Australia. The ecological and
586 economic damage caused by rabbits in Australia was tragic and unparalleled but
587 inadvertently generated a framework that contributed significantly to our
588 understanding of the causes and dynamics of biological invasions. Our results
589 support the importance of the propagule pressure, as many introductions were
590 required before an invasion occurred. However, they suggest that it is not simply the

591 number of individuals and introductions, but also the genetic composition of those
592 individuals that can cause biological invasions. Zenni and Nunēz (2013) noted a lack
593 of studies investigating the genetic differences between successful and unsuccessful
594 invasions. By making this link, we show that while environmental change may have
595 made Australia vulnerable to invasion, it was the genetic makeup of a small batch of
596 wild rabbits that ignited one of the most iconic biological invasions of all time.
597

Material and Methods

Sampling and DNA extraction

We have used a total of 187 individuals in this study. Of this, 179 were wild-caught rabbits collected between 1865 and 2018 in France (n=55), Britain (n=55), mainland Australia (n=62), Tasmania (n=2) and New Zealand (n=5). Additionally, we have sequenced eight domestic rabbits of the following breeds: Belgian Hare, Champagne Silver, English Silver, Fauve de Bourgogne, Flemish Giant, French Angora, Himalayan, and Vienna White. Sequencing data belonging to 153 individuals were obtained from a previous study (29) and 34 new samples were sequenced specifically for this study (Table S1). Original sequence data is available in the Sequence Read Archive under the BioProject ID PRJNA783625.

Library preparation, capture enrichment and sequencing

Extractions of genomic DNA were done using the Qiagen DNAeasy Blood and Tissue Kit (Qiagen, Valencia, CA), following the manufacturer's protocol. Individual barcoded libraries were prepared from the DNA extracts using the KAPA LTP Library Preparation Kit for Illumina platforms (KAPA Biosystems, Boston, USA), following the manufacturer's protocol. After PCR amplification, the libraries were quantified using a qPCR KAPA Library Quantification Kit (KAPA Biosystems, Boston, USA). Two pools of libraries were prepared based on the qPCR quantifications, captured and enriched with a NimbleGen solution-based capture (NimbleGen SeqCap EZ Developer Library, Roche) following the manufacturer's protocol. This capture was used in a previous study (29) and was based on the Ensembl gene annotations (release 2.69) of the OryCun 2.0 rabbit reference genome (34). The total size of the target was 32.10 Mb, which corresponds to 1.17% of the 2.73 Gb rabbit assembly. After capture-enrichment, each pool was independently sequenced in one lane of an Illumina HiSeq 4000 machine using 150bp paired-end reads.

Bioinformatics and variant calling

The quality of the raw sequencing reads was assessed with FastQC (52). Reads were trimmed for low-quality bases and adaptor contamination using Trimmomatic (version 0.32) (53), using the following options: trailing=15 (cut bases at the end of

the read if below a threshold quality of 15), slidingwindow=4:20 (performs a sliding window trimming, cutting once the average quality within the window falls below a threshold of 20), and illuminaclip=TruSeq3-PE.fa:2:20:10:1:true (remove adapter contamination; the values correspond in order to: input fasta file with adapter sequences to be matched, seed mismatches, palindrome clip threshold, simple clip threshold, minimum adapter length and option to keep both reads in case of read-through being detected in paired reads by palindrome mode). Overlapping paired-end reads were merged with Pear (version 0.96) (54) using default parameters. Collapsed and paired-end reads were aligned to the rabbit reference genome OryCun2.0 using bwa-mem(version 0.7.10) and default parameters. PCR duplicates were removed with the *MarkDuplicates* module from Picard Tools, version 1.126 (55).

GATK (version 3.3.0;https://www.broadinstitute.org/GATK) was used for local realignment around indels. Variant calling was carried out for each individual sample using the GATK module *HaplotypeCaller* (version 4.1.8.1) for the target regions with a padding of 300bp around each target, only using reads with a Mapping Quality equal or greater than 30 (56) followed by joint genotyping of all samples with the module *GenotypeGVCFs*. Variants were filtered with *VariantFiltration* module, using the following parameters QD < 2.0, QUAL <30, FS > 60.0, MQ < 40.0, MQRankSum < -12.5, ReadPosRankSum < -8.0, where QD is the variant confidence (from the QUAL field) divided by the unfiltered depth of non-reference samples; FS is the phred-scaled p-value using Fisher's Exact Test to detect strand bias in the reads (the variation seen on only the forward or only the reverse strand); MQ is the Root Mean Square of the mapping quality of the reads across all samples; MQRankSum is the U-based z-approximation from the Mann-Whitney Rank Sum Test for mapping qualities (comparing reads with reference bases versus those with that have an alternate allele); and ReadPosRankSum is the *U*-based z-approximation from the Mann-Whitney Rank Sum Test for the distance from the end of the read for reads with the alternate allele (if the alternate allele is only seen near the ends of reads, this is indicative of error). Only genotypes with a depth of coverage (*DP*) of 10 and a genotype quality (*GQ*) of 30 were kept. VCFtools (57) was used to remove all filtered positions and monomorphic alleles across the entire dataset. Plink (58) was used for making subsets of data for specific populations and selecting different percentages

of missing data or minor allele count thresholds. MapDamage (version 2.06) (59), was used to quantify the damage patterns in historical samples, with downsampling to 100,000 reads, followed by downscaling of the quality score of the potential post-mortem damaged bases.

Population Genetic Analysis

We started by investigating the population structure in rabbit populations with a Principal Component Analysis (PCA) using *Plink2*, version 1.02 (Reference). We only included variants with a genotyping rate >95% and since this analysis included old historical samples, which are enriched for damage-driven mutations, we removed variants that occurred at low frequency (minor allele count =3). A neighbour-joining tree was also constructed using FastMe (version 2.0) (60) based on the proportion of nucleotides that differ between pairs of rabbits (p-distance model) and with 1000 bootstraps. Finally, the ancestry and population structure of rabbit populations was analysed with the program *Admixture*, version 1.23 (31) with *K* values ranging from one to seven.

In analyses where using the distance to the rabbit release point in the property of Thomas Austin (Barwon Park Mansion, Winchelsea, Victoria, Australia; coordinates: -38.224758, 143.995314), the geographic distance was calculated using the individual coordinates of the sample collection sites and the R package Geosphere (61). For samples without exact coordinates, the coordinate of the closest described location was taken.

To construct a genealogy from the full mitochondrial (mtDNA) genome, we used the program BEAST, version 1.10.4 (62). To create genome sequences in a fasta format file, we extracted all reads mapping to the mtDNA using SAMtools, version 1.3 (http://samtools.sourceforge.net). These were converted into a majority-allele fasta file using HTSBOX pileup (https://github.com/lh3/htsbox), where only reads with a mapping quality of 30 and bases with a quality of 30 were kept. After these filters, sites were classified as missing data if they had a read depth of 4x or less. A total of 1,245 bp (out of the 17,245 bp of the European rabbit mtDNA genome) were trimmed at the end of all sequences due to high missing data across samples.

693 Individuals for which more than 20% of sites in the mtDNA sequence were missing
694 were removed from the analysis resulting in a total of 152 individual mtDNA
695 genomes. We included the sequence belonging to the rabbit reference genome,
696 which was derived from a domestic rabbit (GenBank reference: AJ001588).

697 The fasta format files were combined and converted into a nexus format file using
698 AliView (version 1.26), where data was partitioned into five categories: 1st codon
699 position, 2nd codon position, 3rd codon position, control region and others. BEAUti
700 (version 1.10.4) was used to generate an XML file that was used as input for Beast
701 (XML file is available at https://figshare.com/s/78d2b37cd102f3586b8e). The country
702 of origin of each sample was treated as a discrete trait in the phylogenetic analysis
703 (63). Transition rates between countries were estimated with an asymmetric
704 substitution model (i.e. between any pair of countries we estimated two rates
705 corresponding to the two directions of travel). We used a Bayesian Stochastic
706 Search Variable Selection (BSSVS) procedure to identify transitions between
707 countries that are statistically supported (63). The nucleotide substitution model used
708 was the Hasegawa-Kishino-Yano (HKY), with estimated base frequencies and a
709 Gamma site heterogeneity model with 4 categories (64). We used an uncorrelated
710 relaxed clock with a Lognormal Relaxed distribution. Ancestral states were
711 reconstructed for all ancestors and used for plotting the tree. We estimated the
712 number of migration events between different countries using the approach of Minen
713 and Suchard (36). We did four independent runs with different random seeds with a
714 chain length of 100 million steps, sampled at every 1000 steps. Tracer(version 1.7.1)
715 was used to analyse the logs and check for convergence to identify the number of
716 samples to be removed from the start of the MCMC chain as a burn-in. As it is clear
717 from historical records that domestic and European rabbits were introduced into
718 Australasia but not the other way around, we constrained the analysis on this being
719 the case. To do this, we removed any samples from the MCMC chain where the
720 count of state transitions from Australasian populations (Australia, New Zealand or
721 Tasmania) to France, Britain or domestic was greater than zero. The remaining trees
722 were analysed with TreeAnnotator v.1.10.4 to generate a maximum clade credibility
723 tree which was visualised with Figtree (version1.4.4;
724 https://github.com/rambaut/figtree). A median-joining haplotype network of mtDNA

725 genomes was built with PopART with trimming of positions with missing data, leaving
726 a total of 133 segregating sites (version 1.7) (65)

727 To account for uncertainty in genotyping, the site frequency spectrum (SFS), genetic
728 diversity and Tajima's *D* were calculated using the probabilistic framework
729 implemented in ANGSD (version 0.935) (66). We restricted the analysis to protein-
730 coding sequence (based on the annotation version 0.104 of the Orycun2.0 rabbit
731 reference genome) and regions that were covered with exome capture probes (to
732 assure uniform coverage). Unmapped scaffolds from the rabbit reference genome
733 were excluded from the analysis. The total combined size of the regions analysed
734 was 18.87 Mb. Variants were filtered using the following parameters: -baq 1 -
735 remove_bads -C 50 -minMapQ 30 -minQ 30, where -baq 1 performs per-Base
736 Alignment Quality computation to improve accuracy of SNP discovery (67), -C
737 adjusts mapQ for excessive mismatches, minMapQ is the minimum mapping quality
738 of reads and minQ discard bases with a qscore below a threshold. To infer the
739 ancestral state of the variants detected, we used a pseudo-reference genome built
740 with iterative mapping of three different hare species (68). For the SFS analysis, the
741 three populations were down-sampled to 25 individuals, while maximising region
742 representation. Australian individuals from Cattai and Sydney rabbits were excluded
743 from this analysis. Bootstrap confidence intervals on the SFS were obtained by
744 resampling sites with replacement and recalculating the statistics 1000 times. The
745 nucleotide diversity (π) was estimated separately for each chromosome, and the
746 mean was calculated weighting each chromosome equally (69). Bootstrap
747 confidence intervals on the nucleotide diversity estimates were obtained by
748 resampling chromosomes with replacement 1000 times. Chromosome 6 was
749 excluded from calculations since it was an outlier with unusually high genetic
750 diversity. For these analyses, only modern samples were used, to minimise the
751 effect of damage-driven mutations of historical samples, which could bias the
752 estimates of both statistics.

753 We further investigated the historical relationships between the different Australian
754 populations with the program *Treemix* (70). This creates a maximum likelihood tree
755 based on allele frequency correlations between the populations. We used one
756 individual rabbit from the Iberian Peninsula (Spain) as an outgroup. The type of
757 sequencing data generated for this individual was whole-genome and only

overlapping sequences with our exome-target were used for this analysis. A block size (k) of 100 SNPs was used to account for the non-independence of sites due to linkage disequilibrium and ran the 1000 bootstraps by resampling blocks of 100 SNPs. The resulting trees were summarised with the sumtrees function on the package DendroPy (version. 4.1.0) (71). To examine patterns of admixture between populations, we used the three-population statistics (*f*3) of Reich et al. (33), also implemented in *TreeMix*. The tree was computed with the sample size correction turned off due to overcorrection generating branches with zero length.

We explored the impact of the rabbit population expansion on the genetic distance between individuals. For this, we calculated the geographic distance between individuals using the Geosphere package, and the genetic distance using Plink (58) (using the --distance option “square0 1-ibs” that generates an identity-by-state square matrix). To evaluate the statistical significance of the correlation between genetic and geographic distance, we used a Mantel test. To do this, we generated a null distribution of Pearson’s r^2 statistic by permuting the sample locations 1000 times, each time recalculating r^2.

We investigated the occurrence of allele surfing on the front wave of the rabbit expansion throughout mainland Australia by identifying alleles that were absent in the British or domestic population samples and looking at their frequency across Australian populations at different distances from the release point. We used only modern individuals and focused on four different populations, in particular Victoria/NSW, South Australia, Queensland, and Western Australia. For each of these populations, the closest individual to Barwon Park was at a distance of 72 km, 979 km, 1323 km, and 2521 km, respectively. We used a total of seven individuals for each population (Table S1). In Victoria/NSW we sequenced more than seven individuals and therefore selected the seven individuals closest to Barwon Park. Data plots were generated using the R package ggplot2 (72).

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References

1. J. G. Ehrenfeld, Ecosystem Consequences of Biological Invasions. *Annu. Rev. Ecol. Evol. Syst.* **41**, 59–80 (2010).

2. C. Diagne, *et al.*, High and rising economic costs of biological invasions worldwide. *Nature* **592**, 571–576 (2021).

3. R. D. Zenni, M. A. Nuñez, The elephant in the room: the role of failed invasions in understanding invasion biology. *Oikos* **122**, 801–815 (2013).

4. D. Simberloff, The Role of Propagule Pressure in Biological Invasions. *Annu. Rev. Ecol. Evol. Syst.* **40**, 81–102 (2009).

5. J. A. Lau, C. P. terHorst, Causes and consequences of failed adaptation to biological invasions: the role of ecological constraints. *Mol. Ecol.* **24**, 1987–1998 (2015).

6. D. G. Bock, *et al.*, What we still don't know about invasion genetics. *Mol. Ecol.* **24**, 2277–2297 (2015).

7. K. Saltonstall, Cryptic invasion by a non-native genotype of the common reed, Phragmites australis, into North America. *Proc. Natl. Acad. Sci. U. S. A.* **99**, 2445–2449 (2002).

8. C. Hardy, *et al.*, Rabbit mitochondrial DNA diversity from prehistoric to modern times. *J. Mol. Evol.* **40**, 227–237 (1995).

9. G. Queney, N. Ferrand, S. Weiss, F. Mougel, M. Monnerot, Stationary distributions of microsatellite loci between divergent population groups of the European rabbit (Oryctolagus cuniculus). *Mol. Biol. Evol.* **18**, 2169–2178 (2001).

10. J. E. C. Flux, World distribution. In "The European Rabbit: the History and Biology of a Successful Colonizer".(Eds HV Thompson and CM King.) pp. 8--21 (1994).

11. J. E. C. Flux, P. J. Fullagar, World distribution of the Rabbit Oryctolagus funiculus on islands. *Mamm. Rev.* **22**, 151–205 (1992).

12. M. Delibes-Mateos, M. Delibes, P. Ferreras, R. Villafuerte, Key role of European rabbits in the conservation of the Western Mediterranean basin hotspot. *Conserv. Biol.* **22**, 1106–1117 (2008).

13. A. C. Lees, D. J. Bell, A conservation paradox for the 21st century: the European wild rabbitOryctolagus cuniculus, an invasive alien and an endangered native species. *Mammal Review* **38**, 304–320 (2008).

14. J. de Barros, D. do Couto, *Da Asia* (Regia Officina Typografica, 1777).

15. F. Fenner, B. Fantini, Others, *Biological control of vertebrate pests: the history of myxomatosis, an experiment in evolution* (CABI publishing, 1999).

16. K. Myers, I. Parer, D. Wood, B. D. Cooke, The rabbit in Australia. In “The European Rabbit: the History and Biology of a Successful Coloniser”.(Eds HV Thompson and CM King.) pp. 108--157 (1994).

17. B. D. Cooke, Rabbits: manageable environmental pests or participants in new Australian ecosystems? *Wildl. Res.* **39**, 279–289 (2012).

18. W. Gong, J. Sinden, M. Braysher, R. Jones, The economic impacts of vertebrate pests in Australia (2009) (October 11, 2021).

19. J. A. Crooks, Lag times and exotic species: The ecology and management of biological invasions in slow-motion1. *Écoscience* **12**, 316–329 (2005).

20. E. C. Rolls, *They All Ran Wild*, A&R Non-Fiction Classic Edition 1977 (Angus and Robertson, 1969).

21. E. Stodart, I. Parer, *Colonisation of Australia by the rabbit Oryctolagus cuniculus (L.)* (Canberra, CSIRO, 1988).

22. D. Peacock, I. Abbott, The role of quoll (Dasyurus) predation in the outcome of pre-1900 introductions of rabbits (Oryctolagus cuniculus) to the mainland and islands of Australia. *Aust. J. Zool.* **61**, 206–280 (2013).

23. J. A. Gibb, J. M. Williams, The rabbit in New Zealand. In “The European Rabbit: the History and Biology of a Successful Colonizer”.(Eds HV Thompson and CM King.) pp. 158--204 (1994).

24. J. A. Palmer, *Memories of a Riverina Childhood* (New South Wales University Press, 1993).

25. F. G. A. Barnard, Ed., *Gleanings from the Richmond “Australian” 1859-61’* (The Victorian Historical Magazine, 1913).

26. ,*The Bulletin* (John Haynes and J.F. Archibald, 1922).

27. K. R. Zenger, B. J. Richardson, A.-M. Vachot-Griffin, A rapid population expansion retains genetic diversity within European rabbits in Australia. *Mol. Ecol.* **12**, 789–794 (2003).

28. A. Iannella, D. Peacock, P. Cassey, N. Schwensow, Genetic perspectives on the historical introduction of the European rabbit (Oryctolagus cuniculus) to Australia. *Biol. Invasions* **21**, 603–614 (2019).

29. J. M. Alves, *et al.*, Parallel adaptation of rabbit populations to myxoma virus. *Science* **363**, 1319–1326 (2019).

30. F. Depaulis, S. Mousset, M. Veuille, Power of neutrality tests to detect bottlenecks and hitchhiking. *J. Mol. Evol.* **57 Suppl 1**, S190–200 (2003).

31. D. H. Alexander, J. Novembre, K. Lange, Fast model-based estimation of ancestry in unrelated individuals. *Genome Res.* **19**, 1655–1664 (2009).

32. D. J. Lawson, L. van Dorp, D. Falush, A tutorial on how not to over-interpret STRUCTURE and ADMIXTURE bar plots. *Nat. Commun.* **9**, 3258 (2018).

33. D. Reich, K. Thangaraj, N. Patterson, A. L. Price, L. Singh, Reconstructing Indian population history. *Nature* **461**, 489–494 (2009).

34. M. Carneiro, *et al.*, Rabbit genome analysis reveals a polygenic basis for phenotypic change during domestication. *Science* **345**, 1074–1079 (2014).

35. J. M. Alves, *et al.*, Levels and Patterns of Genetic Diversity and Population Structure in Domestic Rabbits. *PLoS One* **10**, e0144687 (2015).

36. V. N. Minin, M. A. Suchard, Counting labeled transitions in continuous-time Markov models of evolution. *J. Math. Biol.* **56**, 391–412 (2008).

37. M. Nei, T. Maruyama, R. Chakraborty, The bottleneck effect and genetic variability in populations. *Evolution* **29**, 1–10 (1975).

38. C. A. Edmonds, A. S. Lillie, L. L. Cavalli-Sforza, Mutations arising in the wave front of an expanding population. *Proc. Natl. Acad. Sci. U. S. A.* **101**, 975–979 (2004).

39. S. Klopfstein, M. Currat, L. Excoffier, The fate of mutations surfing on the wave of a range expansion. *Mol. Biol. Evol.* **23**, 482–490 (2006).

40. I. Mathieson, G. McVean, Demography and the age of rare variants. *PLoS Genet.* **10**, e1004528 (2014).

41. S. Gravel, *et al.*, Demographic history and rare allele sharing among human populations. *Proc. Natl. Acad. Sci. U. S. A.* **108**, 11983–11988 (2011).

42. P. Pereira, J. Teixeira, G. Velo-Antón, Allele surfing shaped the genetic structure of the European pond turtle via colonization and population expansion across the Iberian Peninsula from Africa. *J. Biogeogr.* **45**, 2202–2215 (2018).

43. S. Phillips, K. R. Zenger, B. J. Richardson, Are Sydney rabbits different? *Aust. Zool.* **32**, 49–55 (2002).

44. M. Currat, M. Ruedi, R. J. Petit, L. Excoffier, The hidden side of invasions: massive introgression by local genes. *Evolution* **62**, 1908–1920 (2008).

45. I. Brusini, *et al.*, Changes in brain architecture are consistent with altered fear processing in domestic rabbits. *Proc. Natl. Acad. Sci. U. S. A.* **115**, 7380–7385 (2018).

46. L. Fontanesi, Rabbit Genetic Resources Can Provide Several Animal Models to Explain at the Genetic Level the Diversity of Morphological and Physiological Relevant Traits. *NATO Adv. Sci. Inst. Ser. E Appl. Sci.* **11**, 373 (2021).

47. R. Henriksen, E. Gering, D. Wright, “Feralisation—The Understudied Counterpoint to Domestication” in *Origin and Evolution of Biodiversity*, P. Pontarotti, Ed. (Springer International Publishing, 2018), pp. 183–195.

48. E. Gering, D. Incorvaia, R. Henriksen, D. Wright, T. Getty, Maladaptation in feral and domesticated animals. *Evol. Appl.* **12**, 1274–1286 (2019).

49. J. Sheail, H. V. Thompson, C. M. King, The European Rabbit: The History and Biology of a Successful Colonizer. *The Journal of Applied Ecology* **32**, 254 (1995).

50. B. K. McNab, Minimizing energy expenditure facilitates vertebrate persistence on oceanic islands. *Ecol. Lett.* **5**, 693–704 (2002).

51. C. K. Williams, R. J. Moore, Phenotypic Adaptation and Natural Selection in the Wild Rabbit, Oryctolagus cuniculus, in Australia. *J. Anim. Ecol.* **58**, 495–507 (1989).

52. S. Andrews, FastQC: a quality control tool for high throughput sequence data (2010).

53. A. M. Bolger, M. Lohse, B. Usadel, Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* **30**, 2114–2120 (2014).

54. J. Zhang, K. Kobert, T. Flouri, A. Stamatakis, PEAR: a fast and accurate Illumina Paired-End reAd mergeR. *Bioinformatics* **30**, 614–620 (2014).

55. A. Wysoker, K. Tibbetts, T. Fennell, Picard tools version 1.90. *http://picard. sourceforge. net (Accessed 14 December 2016)* **107**, 308 (2013).

56. R. Poplin, *et al.*, Scaling accurate genetic variant discovery to tens of thousands of samples https:/doi.org/10.1101/201178.

57. P. Danecek, *et al.*, The variant call format and VCFtools. *Bioinformatics* **27**, 2156–2158 (2011).

58. S. Purcell, *et al.*, PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am. J. Hum. Genet.* **81**, 559–575 (2007).

59. H. Jónsson, A. Ginolhac, M. Schubert, P. L. F. Johnson, L. Orlando, mapDamage2.0: fast approximate Bayesian estimates of ancient DNA damage parameters. *Bioinformatics* **29**, 1682–1684 (2013).

60. V. Lefort, R. Desper, O. Gascuel, FastME 2.0: A Comprehensive, Accurate, and Fast Distance-Based Phylogeny Inference Program. *Mol. Biol. Evol.* **32**, 2798–2800 (2015).

61. R. J. Hijmans, E. Williams, C. Vennes, M. R. J. Hijmans, Package “geosphere.” *Spherical trigonometry* **1**, 7 (2017).

62. A. J. Drummond, A. Rambaut, BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evol. Biol.* **7**, 214 (2007).

63. P. Lemey, A. Rambaut, A. J. Drummond, M. A. Suchard, Bayesian phylogeography finds its roots. *PLoS Comput. Biol.* **5**, e1000520 (2009).

64. B. Shapiro, A. Rambaut, A. J. Drummond, Choosing appropriate substitution models for the phylogenetic analysis of protein-coding sequences. *Mol. Biol. Evol.* **23**, 7–9 (2006).

65. J. W. Leigh, D. Bryant, popart : full-feature software for haplotype network construction. *Methods in Ecology and Evolution* **6**, 1110–1116 (2015).

66. T. S. Korneliussen, A. Albrechtsen, R. Nielsen, ANGSD: Analysis of Next Generation Sequencing Data. *BMC Bioinformatics* **15**, 356 (2014).

67. H. Li, Improving SNP discovery by base alignment quality. *Bioinformatics* **27**, 1157–1158 (2011).

68. F. A. Seixas, P. Boursot, J. Melo-Ferreira, The genomic impact of historical hybridization with massive mitochondrial DNA introgression. *Genome Biol.* **19**, 91 (2018).

69. M. Nei, W. H. Li, Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proc. Natl. Acad. Sci. U. S. A.* **76**, 5269–5273 (1979).

70. J. K. Pickrell, J. K. Pritchard, Inference of population splits and mixtures from genome-wide allele frequency data. *PLoS Genet.* **8**, e1002967 (2012).

71. J. Sukumaran, M. T. Holder, DendroPy: a Python library for phylogenetic computing. *Bioinformatics* **26**, 1569–1571 (2010).

72. H. Wickham, *ggplot2: Elegant Graphics for Data Analysis* (Springer, 2016).

Figure Legends

Figure 1 - The colonisation route of the European rabbit from Iberian Peninsula to Australia and New Zealand. Arrows represent introductions. Dashed lines in mainland Australia show the frontier of spread of rabbits across the continent from Thomas Austin property in Barwon Park (based on Stodart and Parer, 1988).

Figure 2 - Genetic diversity of rabbit populations. A) Mean genetic diversity for the different rabbit populations. Dots show mean values where each chromosome is weighted equally. Confidence intervals correspond to the 0.025 and 0.975 quantiles of 100 bootstraps estimations obtained with subsampling and replacement of chromosomes. B) Unfolded allele frequency spectrum (SFS) for France (grey), Britain (blue), and mainland Australia (red). X-axis shows the derived allele frequency. Y-axis shows the number of variants for each category. Confidence intervals correspond to 95% bootstrap confidence intervals obtained by resampling sites with replacement. Analysis only for variants in the protein-coding sequence (CDS) and restricted to 25 individuals per population. The estimates for Australia in both analysis do not include Cattai and Sydney rabbits.

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Figure 4 - Mitochondrial genealogy. A) Maximum clade credibility tree reconstructed with whole mitochondrial genomes, with reconstruction of ancestral geographical location of lineages. Branches and labels are coloured according to the population of origin. Label codes correspond show country and region. Highlighted labels show Cattai and Sydney individuals. B) Median number of migrations inferred into populations in mainland Australia, Tasmania and New Zealand. Error bars are 95% credible intervals. Values in red were included in > 95% of BSSVS models.

Figure 5 - The effect of range expansion on genetic variation and structure. A) Correlation between pairwise genetic and geographic distance for 62 mainland Australian samples. Genetic distance is calculated using only segregating sites. The regression line in red was calculated between all pairs of individuals except Cattai (white) and Sydney (grey). Pairwise comparisons between samples from the same location were not plotted (24 out of 1891 comparisons). (B) Principal components analysis of mainland Australian rabbits excluding Sydney and Cattai. Colour pallet reflects the distance from Barwon Park in kilometres, and symbol shape identifies the population of origin. (C) Genetic diversity in four different regions in mainland Australian. Since our sampling is not uniform, we focused on four distant locations (Victoria/NSW, South Australia, Queensland, and Western Australia) for which we aggregated the seven individuals that were geographically closest in each region. Dots show mean values where each chromosome is weighted equally. 95% confidence intervals are from100 bootstraps estimations obtained by sampling with replacement of chromosomes. (D) Effect of allele surfing in Australia. The frequency of alleles that are absent from domestic and British population across four different mainland Australian populations. Allele frequencies are reported for the same seven rabbits used for genetic diversity estimates (Panel C). Bars are coloured by population.

Figure 6 - British origin of Australian populations. A) Map of the South of Britain. Circles show 17 populations coloured according to the f3 statistics value, reflecting the degree if shared ancestry with Australia. Populations were defined based on the British county of each rabbit. The red triangle marks the location of Baltonsborough village, the residence of the Austin family where the wild rabbits imported to Barwon Park are believed to be originated from. B) Correlation between the proportion of

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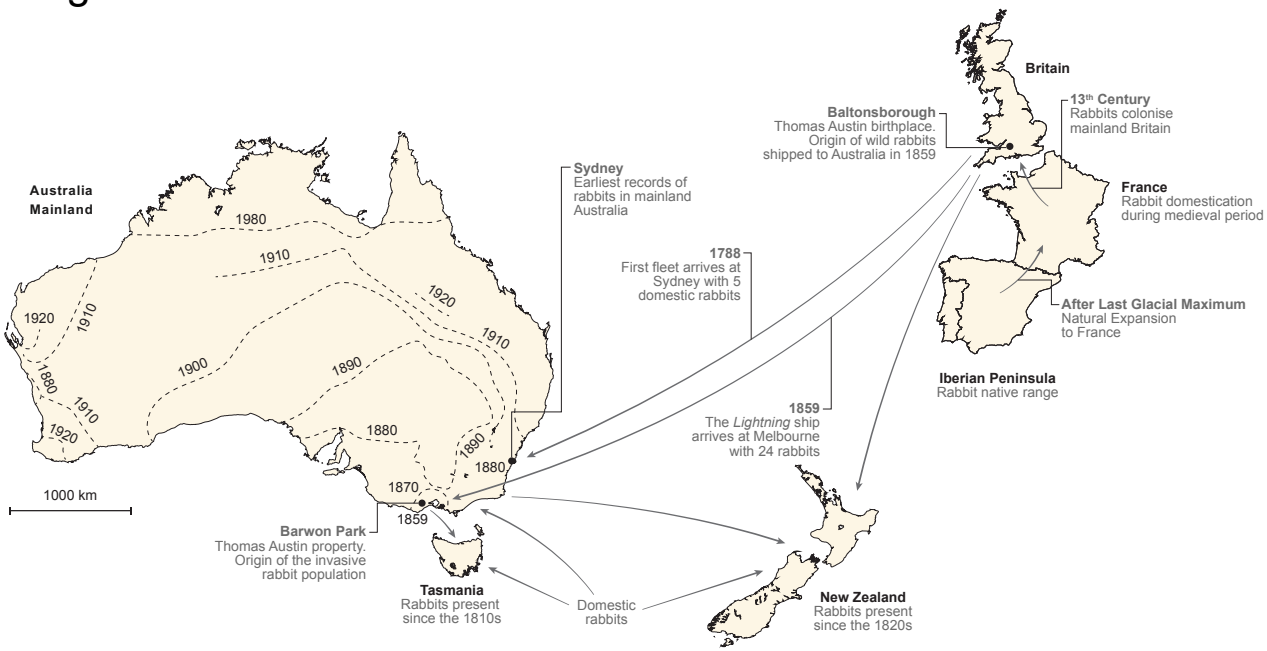
Figures



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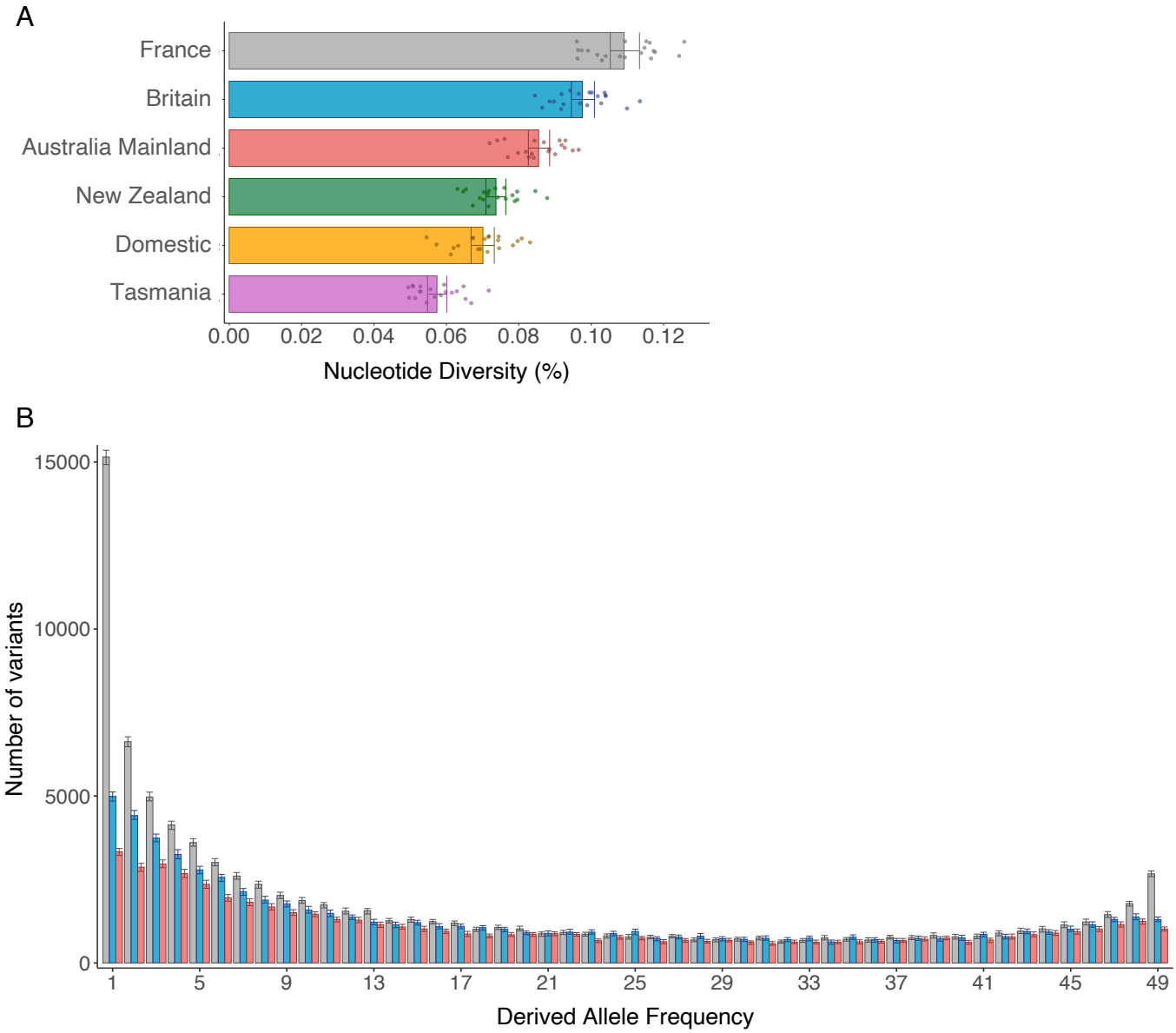

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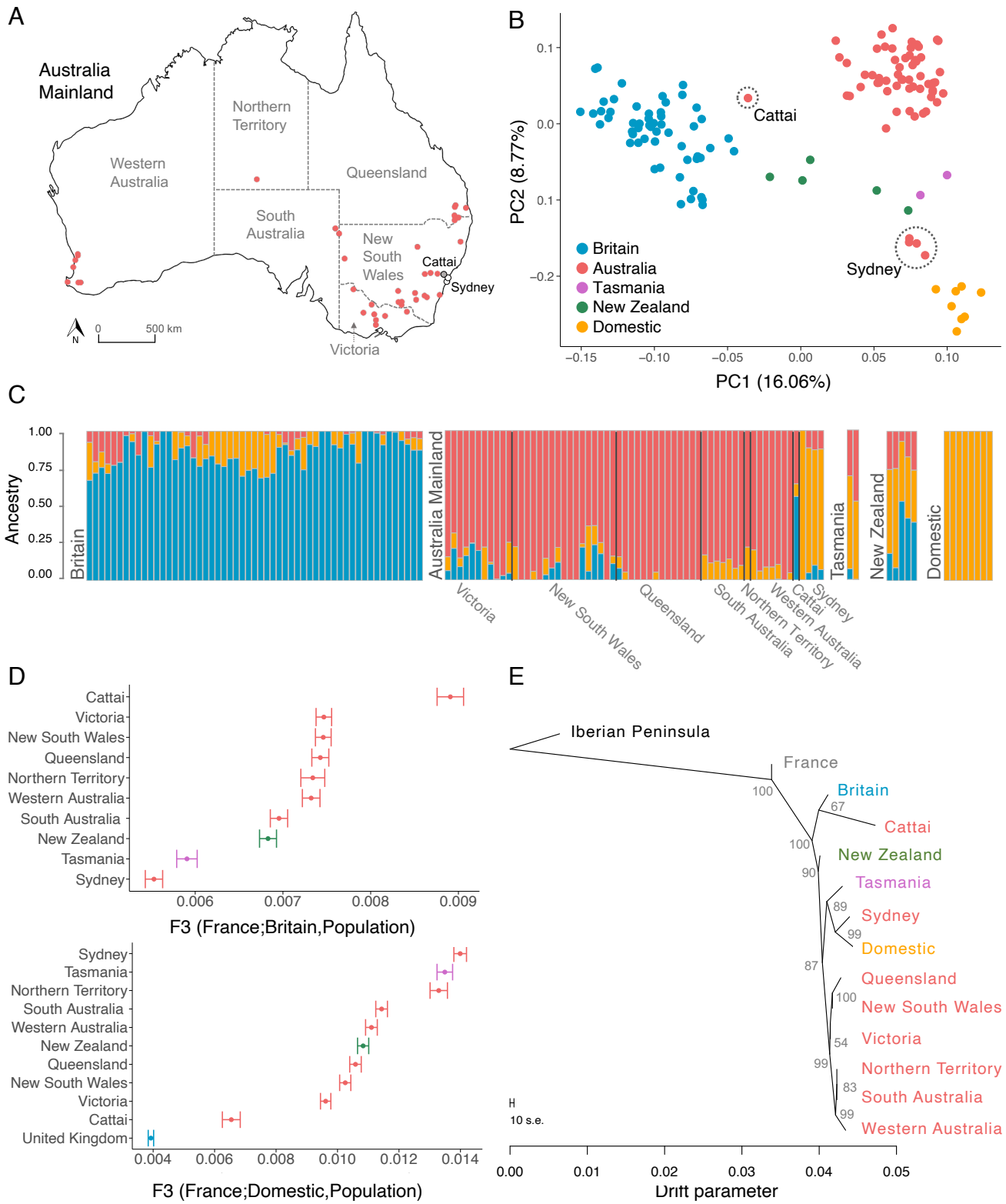

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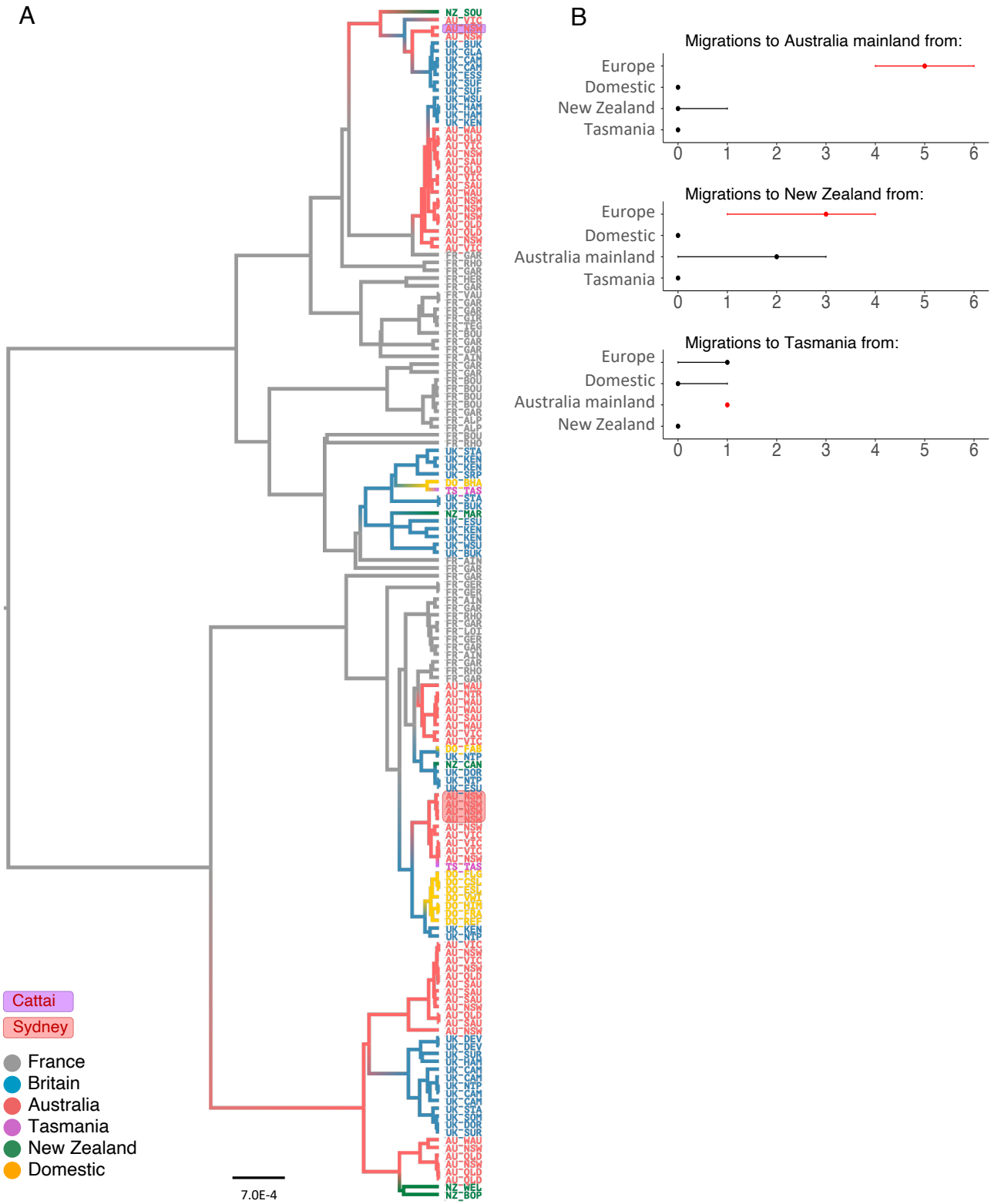

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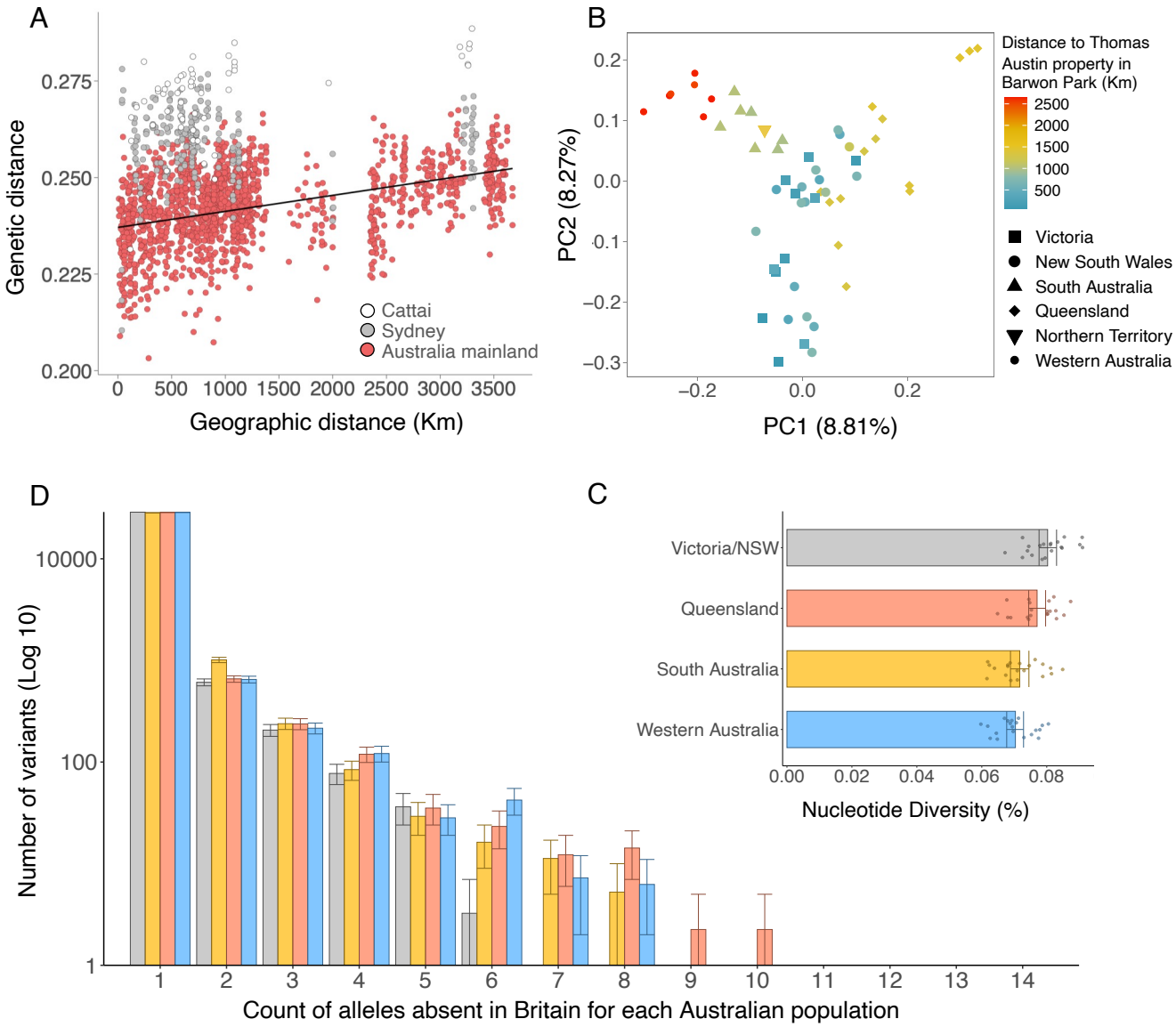

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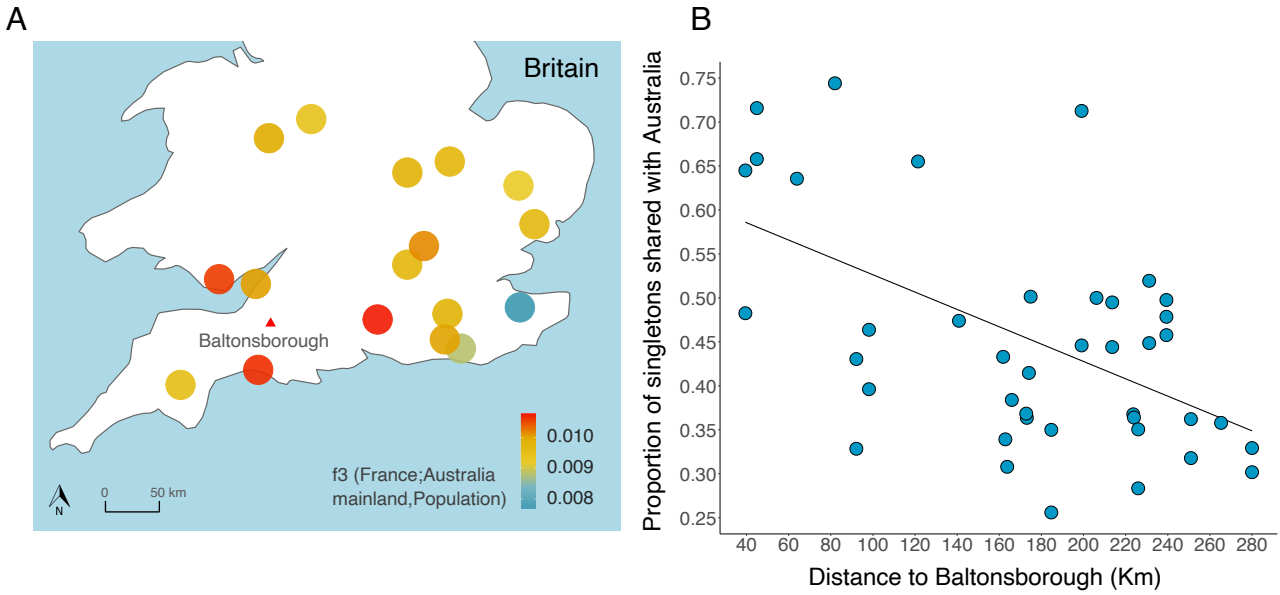

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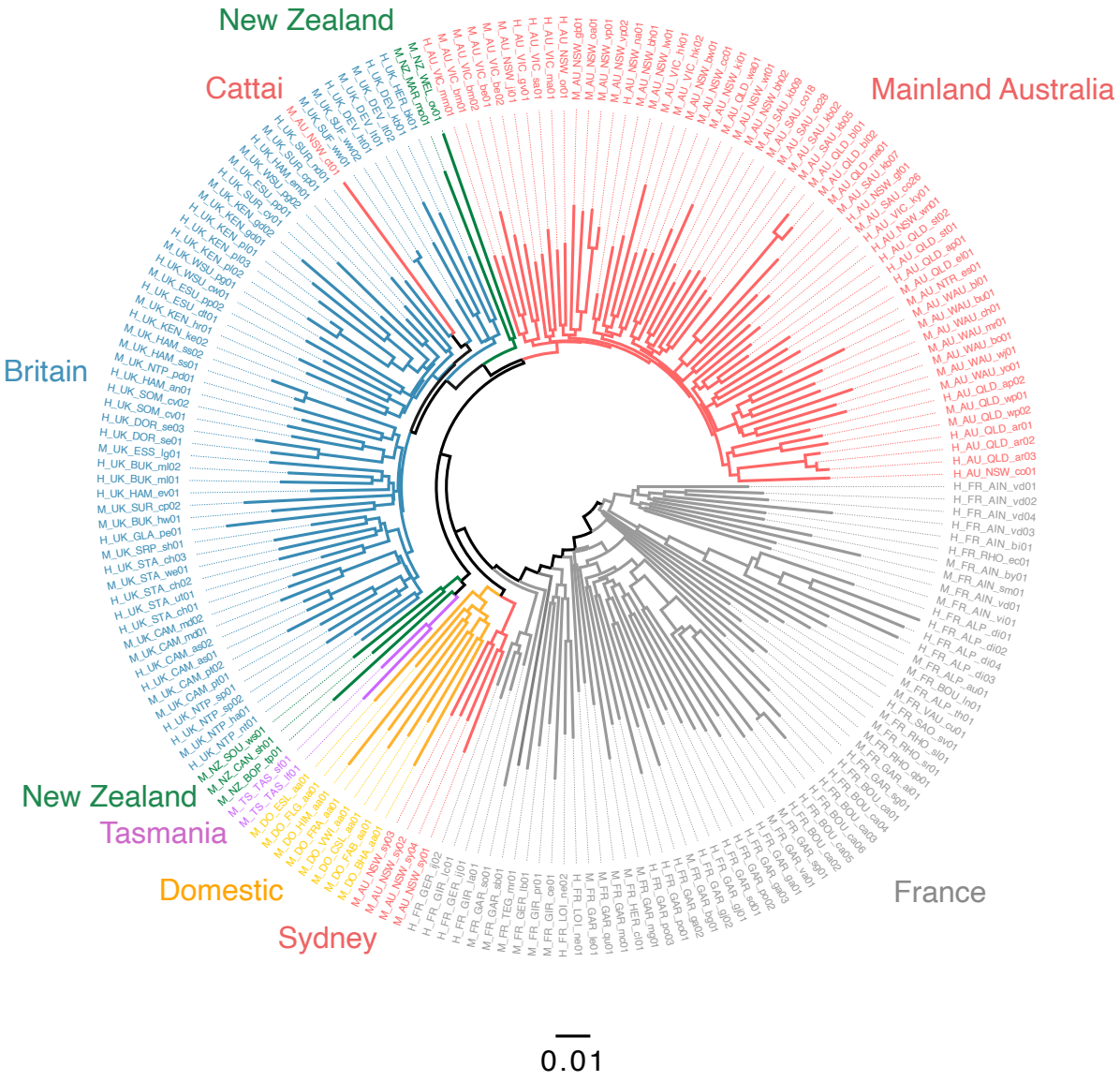
Supplemental Information



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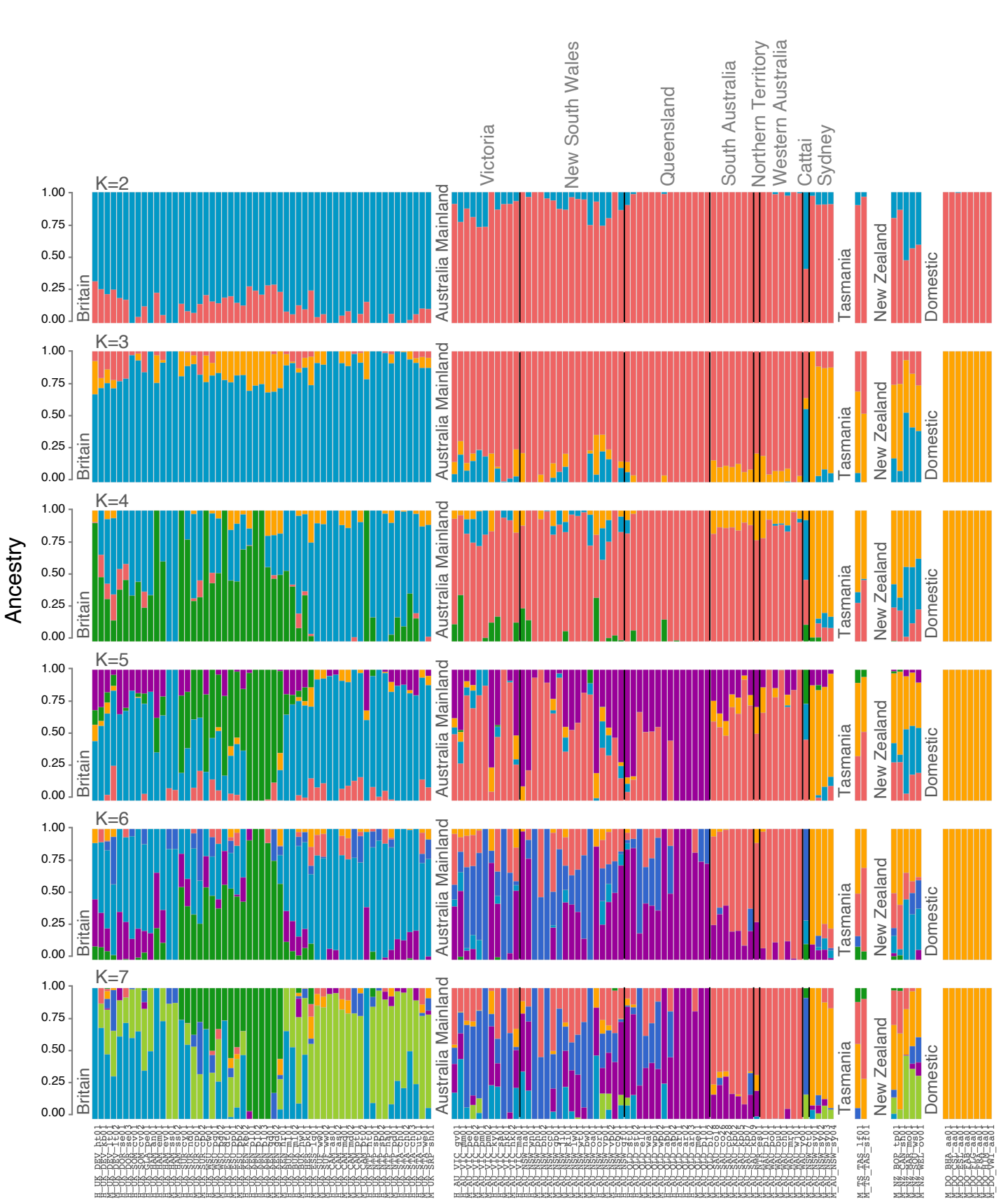

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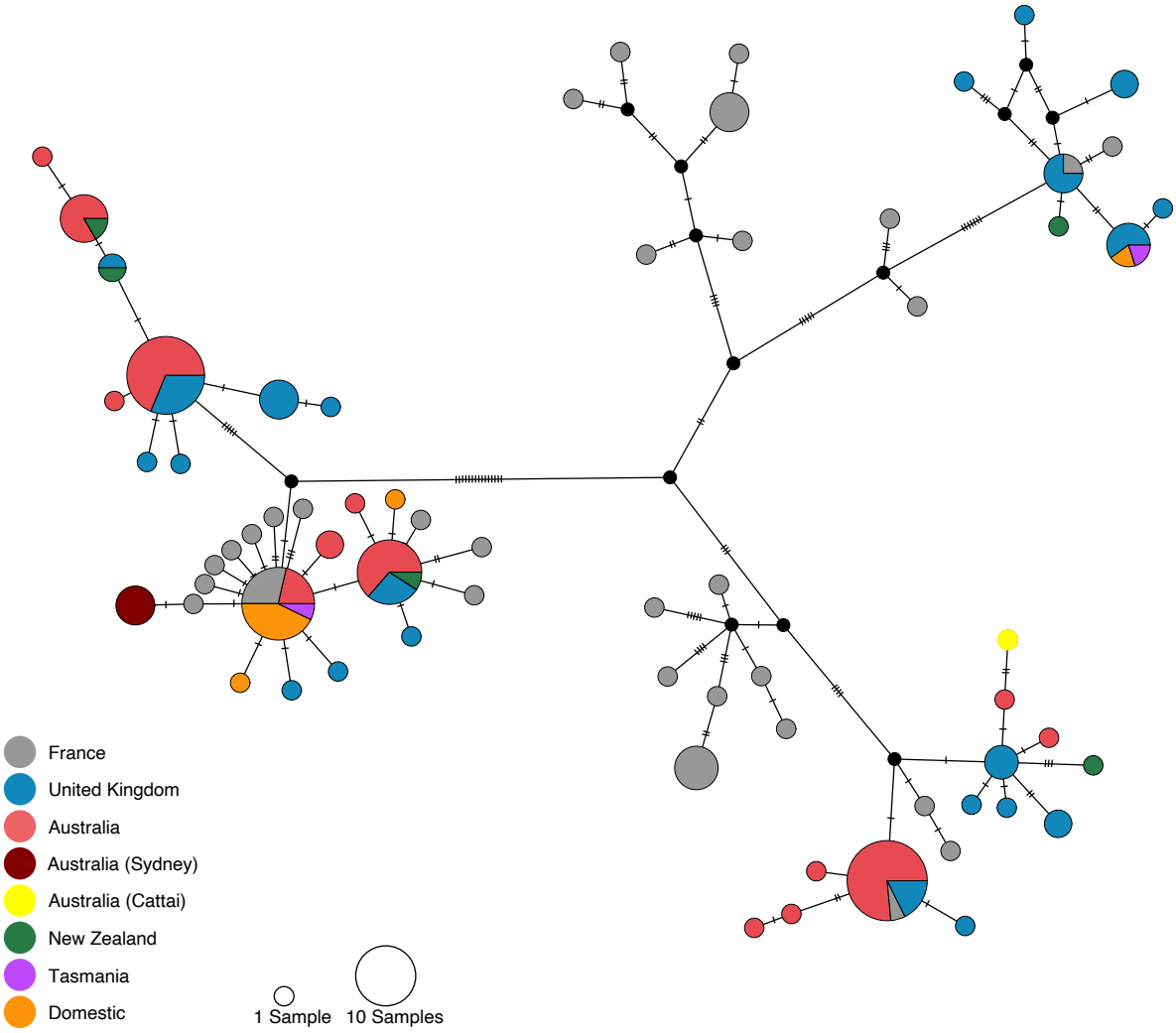

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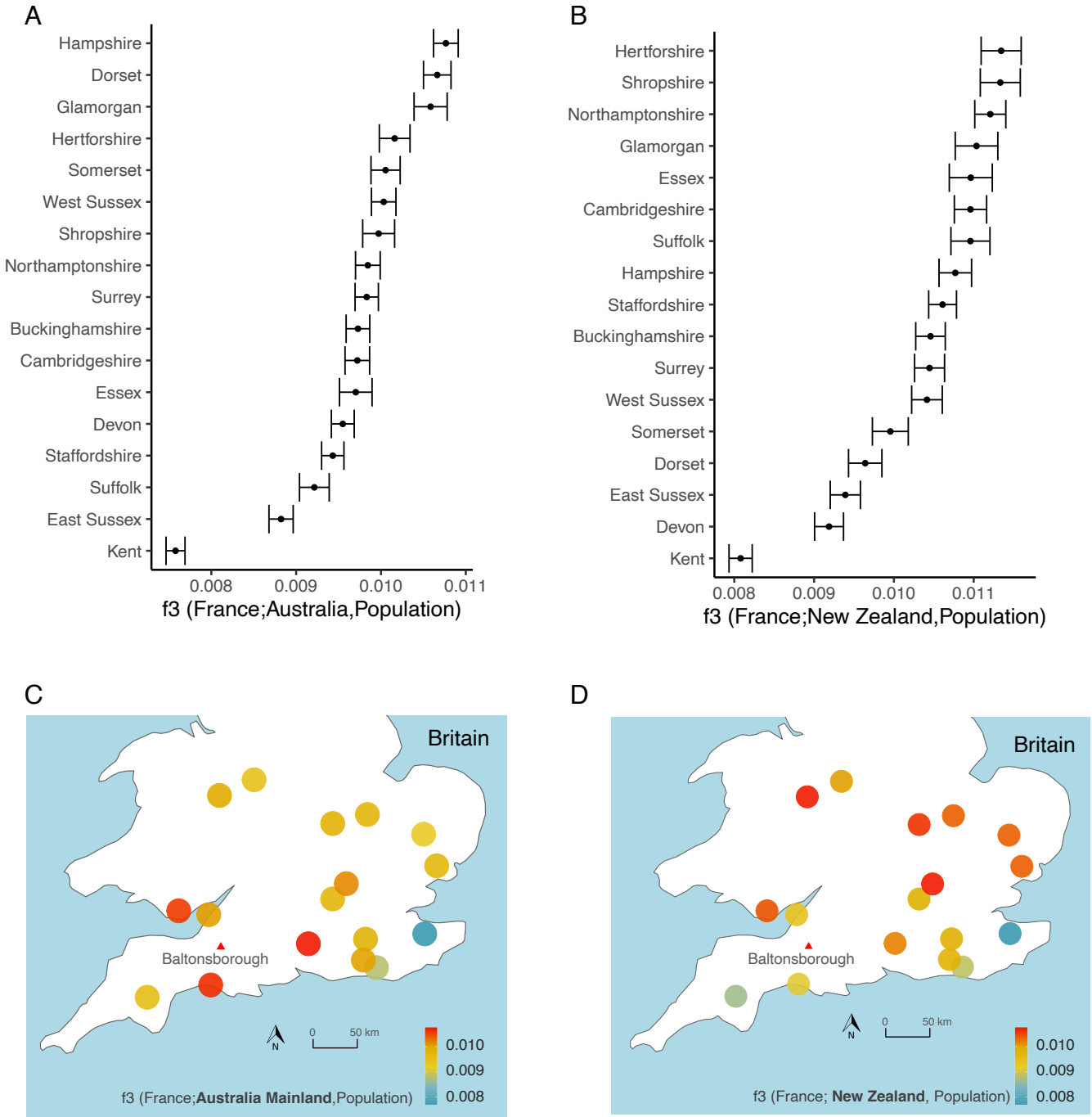

Figure S4 – f3-statistic analysis reflecting the shared genetic drift between mainland Australia (excluding Cattai and Sydney) (A)/New Zealand (B), and 17 British rabbit populations using France as an outgroup. Map of the South of Britain with 17 populations coloured according to the f3 statistics value, reflecting the degree of shared ancestry with Australia (C)/New Zealand (D). Populations were defined based on the British county of each rabbit. The red triangle marks the location of Baltonsborough village, the residence of the Austin family where the wild rabbits imported to Barwon Park are believed to be originated from.

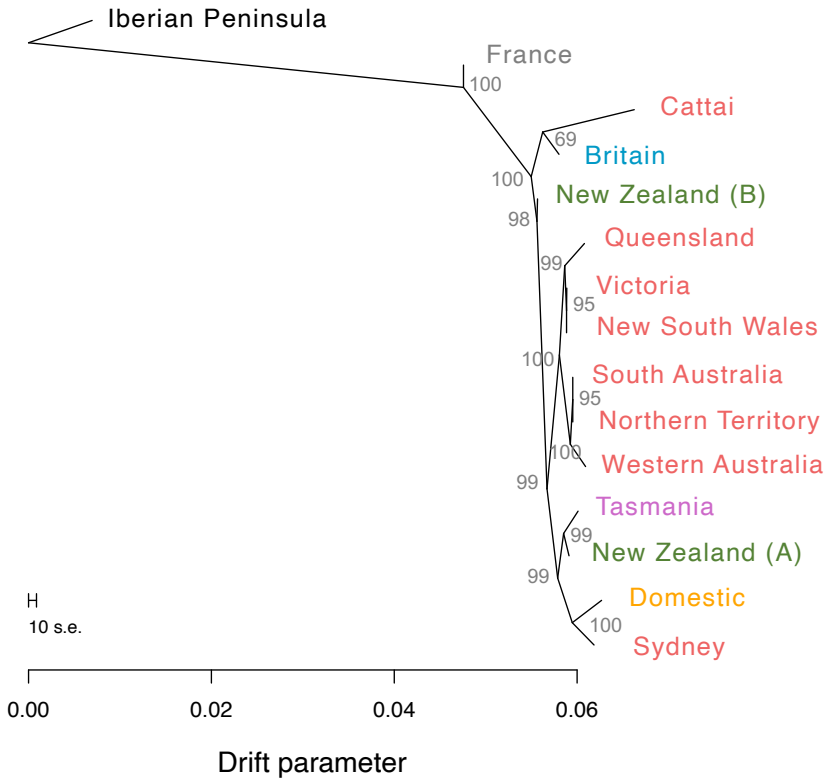

Figure S5 - Historical relationships among populations reconstructed with allele frequency data using the TreeMix program. The branch lengths are proportional to the amount of genetic drift, and the scale bar shows ten times the mean standard error of the entries in the sample covariance matrix. The numbers on branches are percent bootstrap support calculated by resampling blocks of SNPs 1000 times. New Zealand (A) and New Zealand (B) populations correspond to the two clusters of individuals observed in the PCA.

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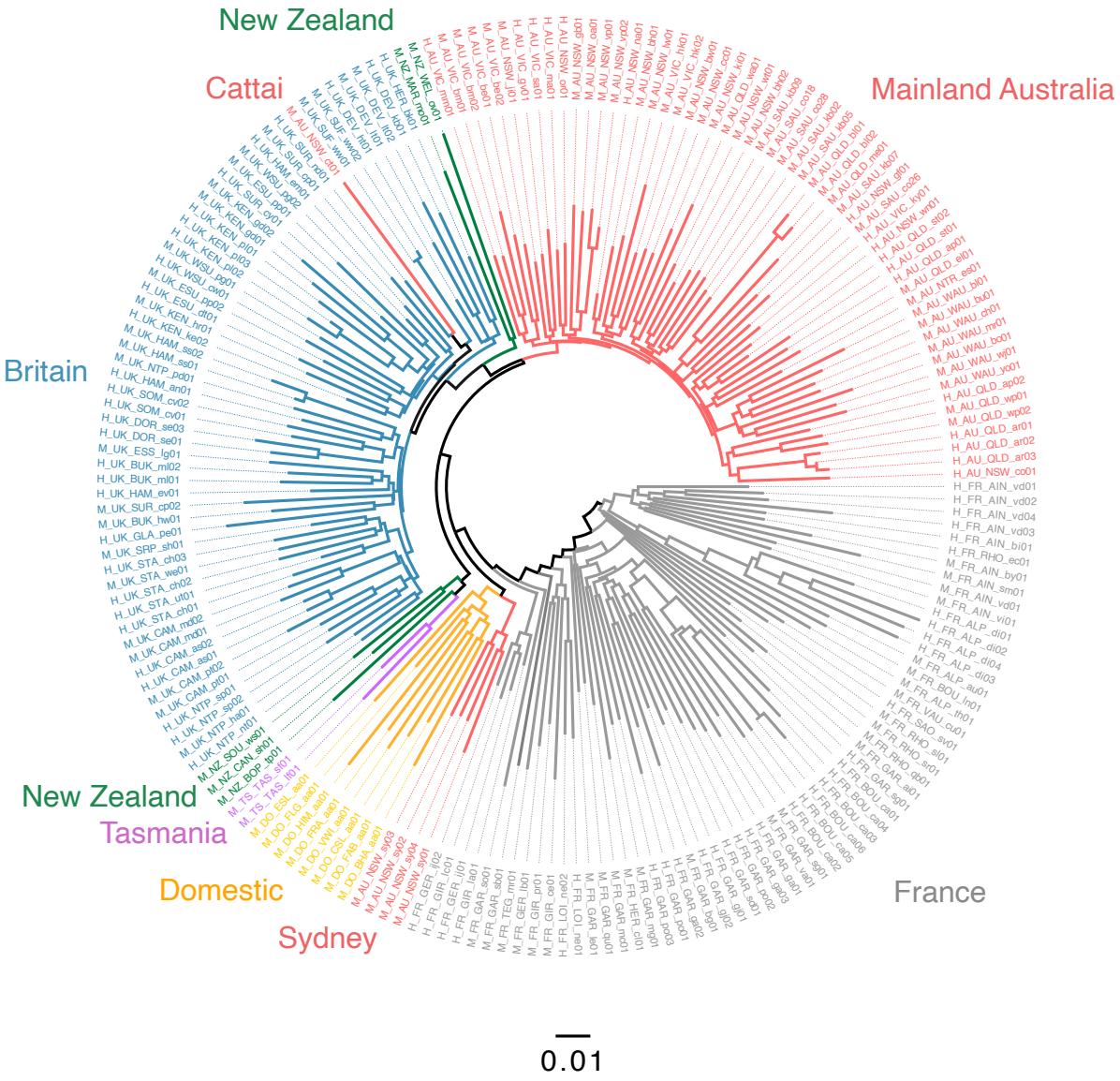

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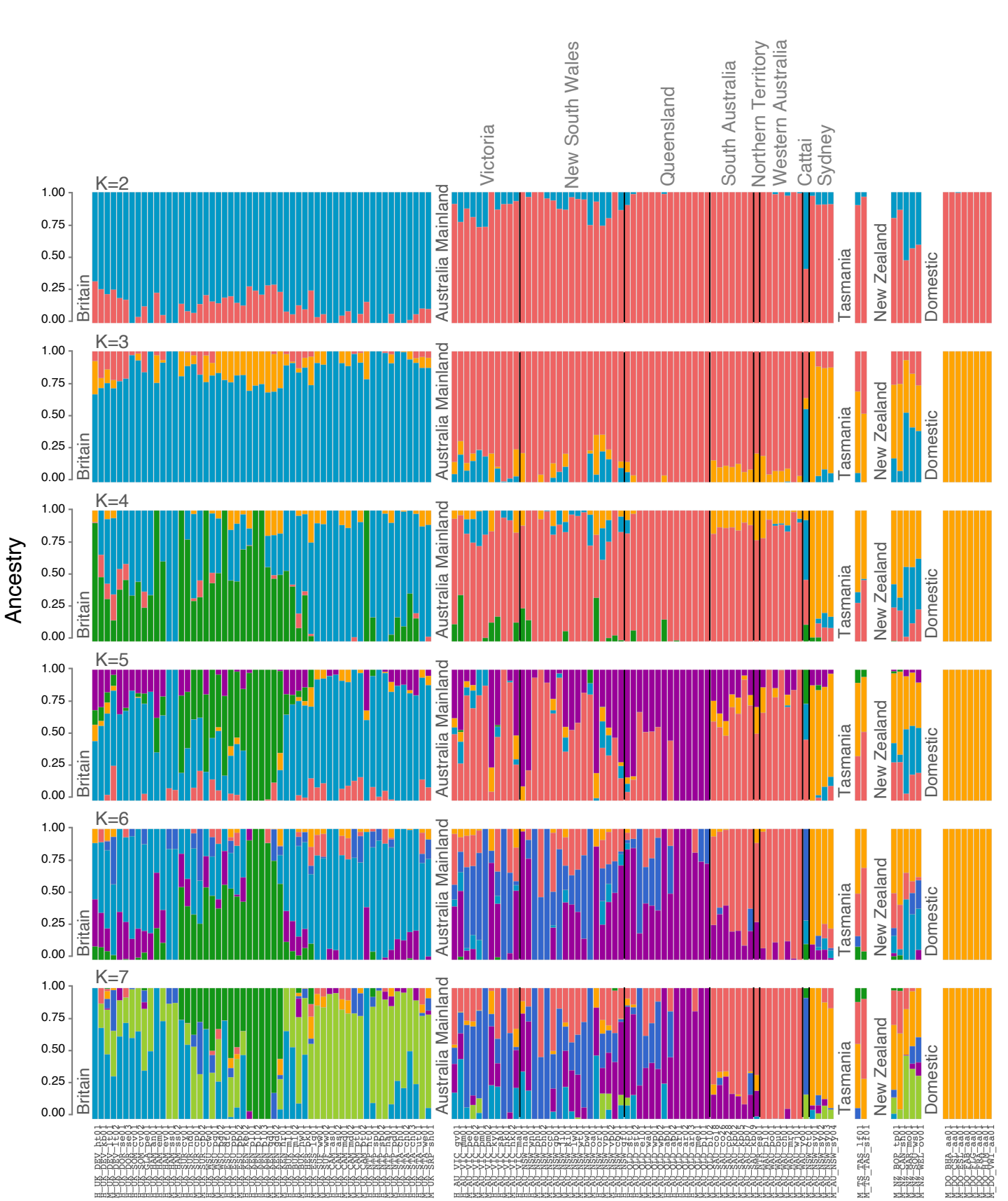

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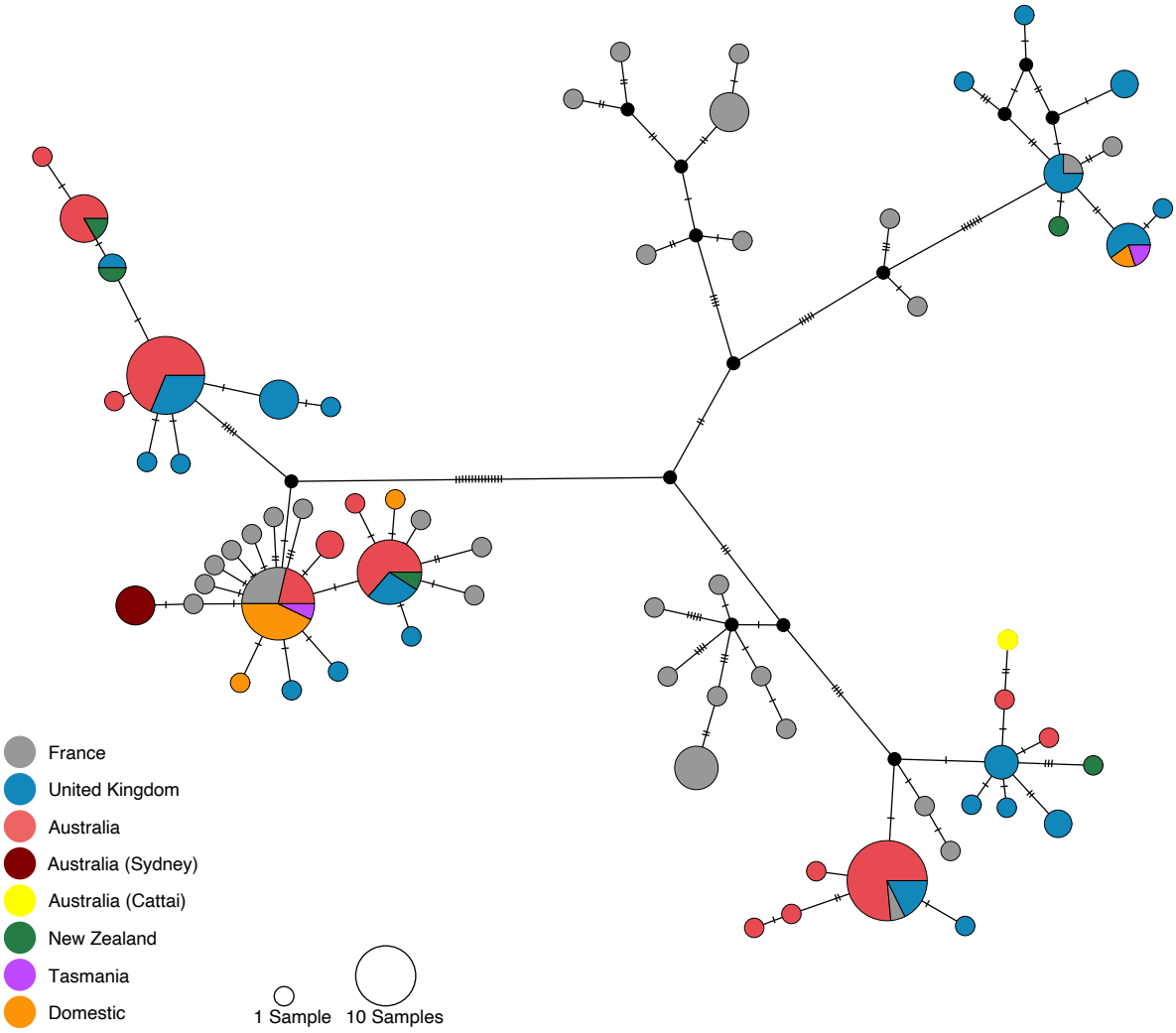

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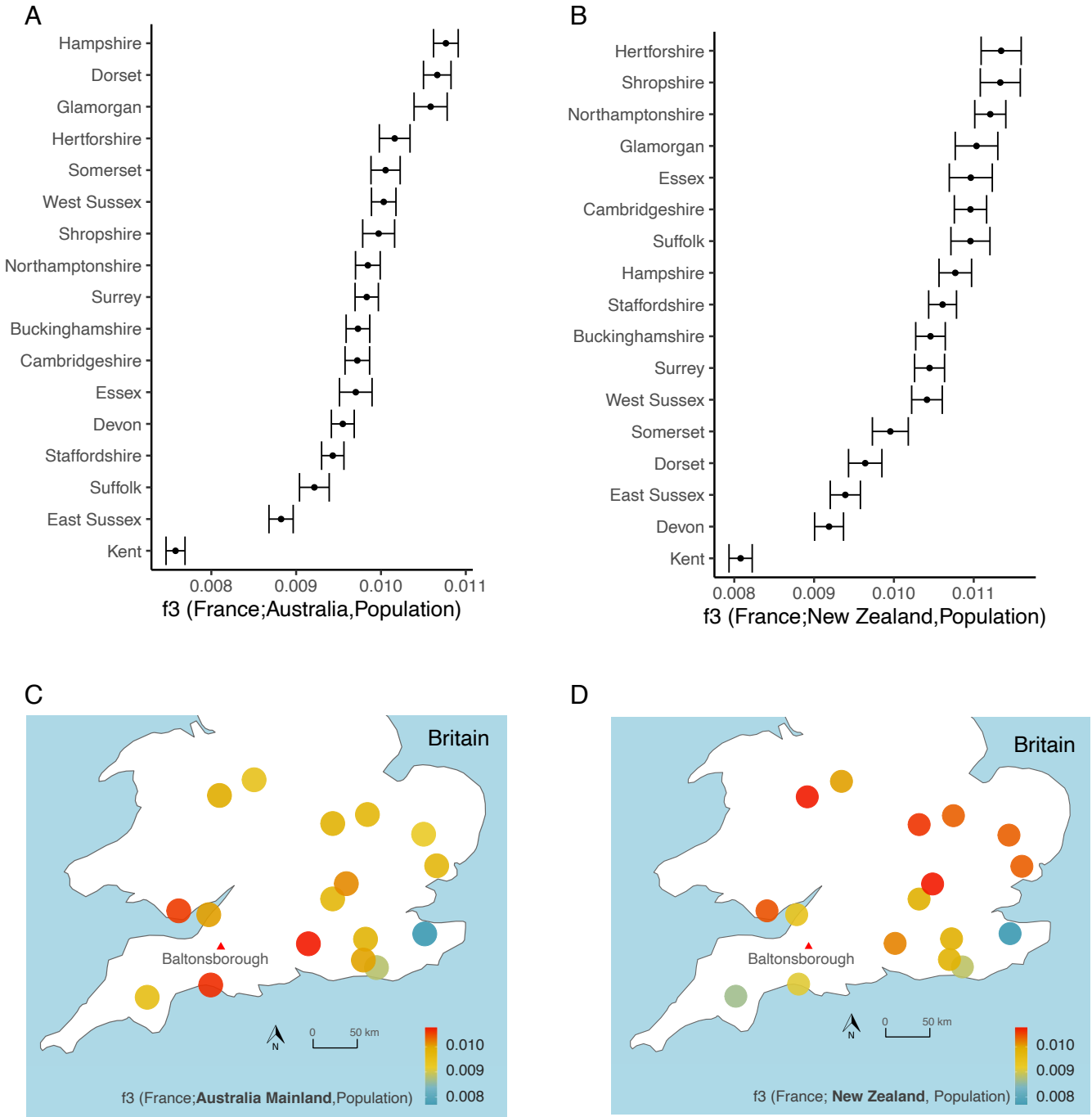

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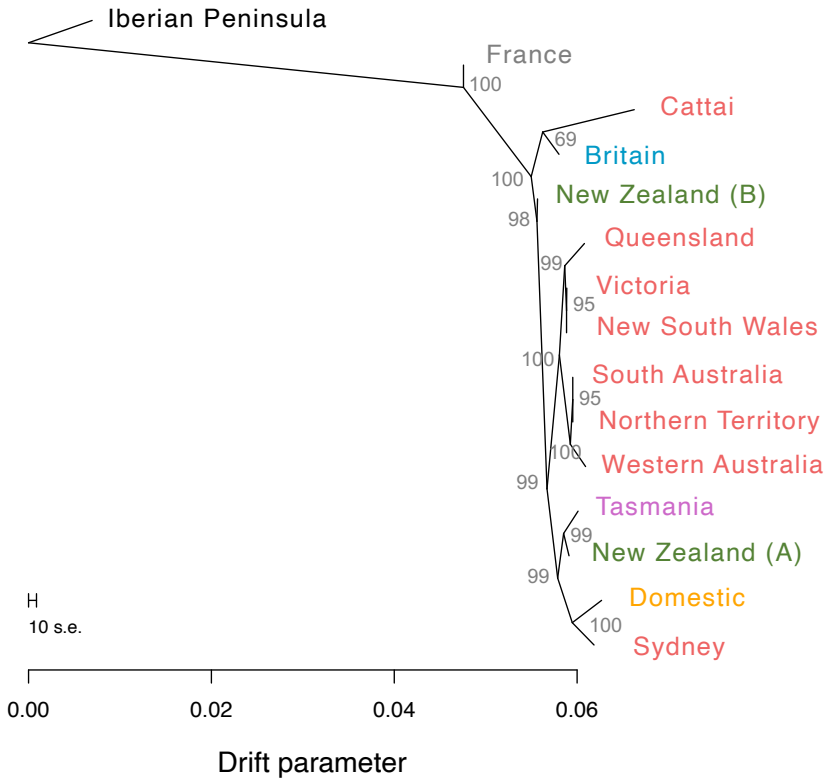

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Table S1 - List of all samples used in this study.

Sample	Published data	Year	Origin	State/County	Locality/Breed	Lat	Long	Representative of Population
M_AU_NSW_bh01	Alves et al 2019	2012	Australia	New South Wales	Broken Hill	-31.956	141.465	-
M_AU_NSW_bh02	Alves et al 2019	2012	Australia	New South Wales	Broken Hill	-31.956	141.465	-
M_AU_NSW_bw01	Alves et al 2019	2013	Australia	New South Wales	Blowering	-35.506	148.273	-
M_AU_NSW_cc01	Alves et al 2019	2013	Australia	New South Wales	Coolac	-34.927	148.166	-
M_AU_NSW_ct01	Alves et al 2019	2007	Australia	New South Wales	Cattai National park	-33.320	150.907	-
M_AU_NSW_gb01	Alves et al 2019	2013	Australia	New South Wales	Goombargana	-35.702	146.583	VIC/NSW
M_AU_NSW_ji01	Alves et al 2019	2013	Australia	New South Wales	Jindera	-35.950	146.881	VIC/NSW
M_AU_NSW_ki01	Alves et al 2019	2013	Australia	New South Wales	King's	-35.676	146.898	VIC/NSW
M_AU_NSW_lw01	Alves et al 2019	2010	Australia	New South Wales	Little Whiskters Road (Carwoola)	-35.292	149.300	-
M_AU_NSW_oa01	Alves et al 2019	2007	Australia	New South Wales	Oakey Creek (near Bathurst)	-33.255	150.300	-
M_AU_NSW_sy01	New data	2018	Australia	New South Wales	Avalon	-33.607	151.325	-
M_AU_NSW_sy02	New data	2018	Australia	New South Wales	Avalon	-33.607	151.325	-
M_AU_NSW_sy03	New data	2018	Australia	New South Wales	Avalon	-33.607	151.325	-
M_AU_NSW_sy04	New data	2018	Australia	New South Wales	East Lake Golf Course	-33.926	151.221	-
M_AU_NSW_vp01	Alves et al 2019	2007	Australia	New South Wales	Valpine (Near Bathurst)	-33.187	149.641	-
M_AU_NSW_vp02	Alves et al 2019	2007	Australia	New South Wales	Valpine (Near Bathurst)	-33.187	149.641	-
M_AU_NSW_wt01	Alves et al 2019	2007	Australia	New South Wales	Wattagan (near Canberra)	-35.152	149.072	-
M_AU_NTR_es01	New data	2011	Australia	Northern Australia	Erldunda Station	-25.120	133.114	-
M_AU_QLD_bl01	Alves et al 2019	2013	Australia	Queensland	Blue Mountain Heights	-27.502	151.952	QLD
M_AU_QLD_bl02	Alves et al 2019	2013	Australia	Queensland	Blue Mountain Heights	-27.502	151.952	QLD
M_AU_QLD_el01	Alves et al 2019	2013	Australia	Queensland	Elbow valley	-28.438	152.205	QLD
M_AU_QLD_me01	Alves et al 2019	2013	Australia	Queensland	Merrits	-27.362	152.028	QLD
M_AU_QLD_wa01	Alves et al 2019	2013	Australia	Queensland	Warwick	-28.368	151.966	QLD
M_AU_QLD_wp01	Alves et al 2019	2013	Australia	Queensland	Washpool	-28.240	151.917	QLD

M_AU_QLD_wp02	Alves et al 2019	2013	Australia	Queensland	Washpool	-28.240	151.917	QLD
M_AU_SAU_co18	New data	2018	Australia	South Australia	Coonanna	-29.796	140.947	SAU
M_AU_SAU_co26	New data	2018	Australia	South Australia	Coonanna	-29.796	140.947	SAU
M_AU_SAU_co28	New data	2018	Australia	South Australia	Coonanna	-29.796	140.947	SAU
M_AU_SAU_kb02	New data	2018	Australia	South Australia	Kain Bore	-29.349	140.589	SAU
M_AU_SAU_kb05	New data	2018	Australia	South Australia	Kain Bore	-29.349	140.589	SAU
M_AU_SAU_kb07	New data	2018	Australia	South Australia	Kain Bore	-29.349	140.589	SAU
M_AU_SAU_kb09	New data	2018	Australia	South Australia	Kain Bore	-29.349	140.589	SAU
M_AU_VIC_be01	Alves et al 2019	2009	Australia	Victoria	Bendigo	-36.758	144.284	VIC/NSW
M_AU_VIC_be02	Alves et al 2019	2009	Australia	Victoria	Bendigo	-36.758	144.284	VIC/NSW
M_AU_VIC_bm01	Alves et al 2019	2009	Australia	Victoria	Bacchus Marsh	-37.676	144.439	VIC/NSW
M_AU_VIC_bm02	Alves et al 2019	2009	Australia	Victoria	Bacchus Marsh	-37.676	144.439	VIC/NSW
M_AU_VIC_hk01	Alves et al 2019	2009	Australia	Victoria	Hattah Kulkyne National Park	-34.608	142.303	-
M_AU_VIC_hk02	Alves et al 2019	2009	Australia	Victoria	Hattah Kulkyne National Park	-34.608	142.303	-
M_AU_WAU_bl01	New data	2017	Australia	Western Australia	Bibra Lake	-32.091	115.831	WAU
M_AU_WAU_bo01	New data	2017	Australia	Western Australia	Bouvard	-32.690	115.642	WAU
M_AU_WAU_bu01	New data	2016	Australia	Western Australia	Bullsbrook	-31.692	116.071	WAU
M_AU_WAU_ch01	New data	2016	Australia	Western Australia	Chittering	-31.570	116.069	WAU
M_AU_WAU_mr01	New data	2016	Australia	Western Australia	Margaret River	-33.975	115.101	WAU
M_AU_WAU_wj01	New data	2016	Australia	Western Australia	Winnejup	-34.017	116.334	WAU
M_AU_WAU_yo01	New data	2016	Australia	Western Australia	Yornup	-34.021	116.123	WAU
H_AU_NSW_co01	Alves et al 2019	1902	Australia	New South Wales	Coonamble	-30.950	148.400	-
H_AU_NSW_gf01	Alves et al 2019	1924	Australia	New South Wales	Guy Fawkes District	-30.500	152.500	-
H_AU_NSW_na01	Alves et al 2019	1900	Australia	New South Wales	Narrandera	-34.750	146.550	-
H_AU_NSW_or01	Alves et al 2019	1956	Australia	New South Wales	Orange	-33.283	149.100	-
H_AU_NSW_wn01	Alves et al 2019	1919	Australia	New South Wales	Wandandian	-35.092	150.508	-
H_AU_QLD_ap01	Alves et al 2019	1947	Australia	Queensland	Acacia Plateau	-28.417	152.300	-

H_AU_QLD_ap02	Alves et al 2019	1947	Australia	Queensland	Acacia Plateau	-28.417	152.300	-
H_AU_QLD_ar01	Alves et al 2019	1947	Australia	Queensland	Acacia Ridge	-27.583	153.017	-
H_AU_QLD_ar02	Alves et al 2019	1947	Australia	Queensland	Acacia Ridge	-27.583	153.017	-
H_AU_QLD_ar03	Alves et al 2019	1947	Australia	Queensland	Acacia Ridge	-27.583	153.017	-
H_AU_QLD_st01	Alves et al 2019	1941	Australia	Queensland	Stanthorpe area	-28.650	151.933	-
H_AU_QLD_st02	Alves et al 2019	1941	Australia	Queensland	Stanthorpe area	-28.650	151.933	-
H_AU_VIC_gv01	Alves et al 2019	1903	Australia	Victoria	Goulburn Valley	-36.895	145.314	-
H_AU_VIC_ky01	Alves et al 2019	1911	Australia	Victoria	Kyneton	-37.248	144.454	-
H_AU_VIC_ma01	Alves et al 2019	1910	Australia	Victoria	Macorna	-35.917	144.033	-
H_AU_VIC_mm01	Alves et al 2019	1914	Australia	Victoria	Mitta Mitta	-36.533	147.383	-
H_AU_VIC_sa01	Alves et al 2019	1909	Australia	Victoria	St Arnaud	-36.617	143.250	-
M_DO_BHA_aa01	New data	-	Domestic	-	Belgian Hare	-	-	-
M_DO_CSL_aa01	New data	-	Domestic	-	Champagne Silver	-	-	-
M_DO_ESL_aa01	New data	-	Domestic	-	English Silver	-	-	-
M_DO_FAB_aa01	New data	-	Domestic	-	Fauve de Bourgogne	-	-	-
M_DO_FLG_aa01	New data	-	Domestic	-	Flemish Giant	-	-	-
M_DO_FRA_aa01	New data	-	Domestic	-	French Angora	-	-	-
M_DO_HIM_aa01	New data	-	Domestic	-	Himalayan	-	-	-
M_DO_VWI_aa01	New data	-	Domestic	-	Vienna White	-	-	-
M_FR_AIN_by01	Alves et al 2019	2005	France	Ain	Beynost	45.840	5.002	-
M_FR_AIN_sm01	Alves et al 2019	2005	France	Ain	St Marcel	45.948	4.991	-
M_FR_AIN_vd01	Alves et al 2019	2005	France	Ain	Villars les Dombes	46.002	5.037	-
M_FR_AIN_vi01	Alves et al 2019	2005	France	Ain	Villieu	45.920	5.222	-
M_FR_ALP_au01	Alves et al 2019	2005	France	Alpes-de-Haute-Provence	Aubignosc	44.129	5.970	-
M_FR_ALP_th01	Alves et al 2019	2005	France	Alpes-de-Haute-Provence	Thèze	44.319	5.922	-
M_FR_BOU_ln01	Alves et al 2019	2005	France	Bouches-du-Rhône	Lançon de Provence	43.592	5.127	-
M_FR_GAR_ai01	Alves et al 2019	2005	France	Gard	Aigremont	43.966	4.123	-

M_FR_GAR_bg01	Alves et al 2019	2005	France	Gard	Bagard	44.073	4.052	-
M_FR_GAR_le01	Alves et al 2019	2005	France	Gard	Le Cailar	43.676	4.236	-
M_FR_GAR_mc01	Alves et al 2019	2005	France	Gard	Mejannes le Clap	44.225	4.349	-
M_FR_GAR_mg01	Alves et al 2019	2005	France	Gard	Marguerittes	43.863	4.433	-
M_FR_GAR_qu01	Alves et al 2019	2005	France	Gard	Quissac	43.911	3.999	-
M_FR_GAR_sb01	Alves et al 2019	2005	France	Gard	St Ambroix	44.260	4.196	-
M_FR_GAR_sg01	Alves et al 2019	2005	France	Gard	St Gilles	43.676	4.448	-
M_FR_GAR_so01	Alves et al 2019	2005	France	Gard	Souvignargues	43.816	4.122	-
M_FR_GAR_va01	Alves et al 2019	2005	France	Gard	Vauvert	43.693	4.278	-
M_FR_GER_lb01	Alves et al 2019	2005	France	Gers	Labastide-Saves	43.520	0.981	-
M_FR_GIR_ce01	Alves et al 2019	2002	France	Gironde	Cerons	44.631	-0.338	-
M_FR_GIR_pr01	Alves et al 2019	2002	France	Gironde	Preignac	44.586	-0.296	-
M_FR_HER_cl01	Alves et al 2019	2005	France	Hérault	Claret	43.862	3.906	-
M_FR_RHO_qb01	Alves et al 2019	2002	France	Rhône	Quincie en Beaujolais	46.119	4.615	-
M_FR_RHO_sl01	Alves et al 2019	2002	France	Rhône	St Genis Laval	45.692	4.793	-
M_FR_RHO_sr01	Alves et al 2019	2002	France	Rhône	St Germain-Nuelles	45.850	4.611	-
M_FR_TEG_mr01	Alves et al 2019	2005	France	Tarn-et-Garonne	Marignac	43.844	0.912	-
M_FR_VAU_cu01	Alves et al 2019	2012	France	Vaucluse	Caumont sur Durance	43.894	4.943	-
H_FR_AIN_bi01	Alves et al 2019	1925	France	Ain	Birieux	45.953	5.039	-
H_FR_AIN_vd01	Alves et al 2019	1928	France	Ain	Villars-les-Dombes	46.002	5.029	-
H_FR_AIN_vd02	Alves et al 2019	1908	France	Ain	Villars-les-Dombes	46.002	5.029	-
H_FR_AIN_vd03	Alves et al 2019	1925	France	Ain	Villars-les-Dombes	46.002	5.029	-
H_FR_AIN_vd04	Alves et al 2019	1925	France	Ain	Villars-les-Dombes	46.002	5.029	-
H_FR_ALP_di01	Alves et al 2019	1908	France	Alpes-de-Haute-Provence	Digne, Basses Alpes	44.092	6.236	-
H_FR_ALP_di02	Alves et al 2019	1908	France	Alpes-de-Haute-Provence	vers Digne, Basses Alpes	44.092	6.236	-
H_FR_ALP_di03	Alves et al 2019	1908	France	Alpes-de-Haute-Provence	vers Digne, Basses Alpes	44.092	6.236	-
H_FR_ALP_di04	Alves et al 2019	1908	France	Alpes-de-Haute-Provence	vers Digne, Basses Alpes	44.092	6.236	-
H_FR_BOU_ca01	Alves et al 2019	1956	France	Bouches-du-Rhône	Camargue	43.590	4.384	-
H_FR_BOU_ca02	Alves et al 2019	1952	France	Bouches-du-Rhône	Camargue, Tour du valat	43.590	4.384	-
H_FR_BOU_ca03	Alves et al 2019	1952	France	Bouches-du-Rhône	Camargue, Tour du valat	43.590	4.384	-
H_FR_BOU_ca04	Alves et al 2019	1953	France	Bouches-du-Rhône	Camargue, Tour du valat	43.590	4.384	-
H_FR_BOU_ca05	Alves et al 2019	1953	France	Bouches-du-Rhône	Camargue, Tour du valat	43.590	4.384	-

H_FR_BOU_ca06	Alves et al 2019	1952	France	Bouches-du-Rhône	Camargue, Tour du valat	43.590	4.384	-
H_FR_GAR_ga01	Alves et al 2019	1907	France	Gard	Gard	43.945	4.151	-
H_FR_GAR_ga02	Alves et al 2019	1932	France	Gard	Gard	43.945	4.151	-
H_FR_GAR_ga03	Alves et al 2019	1907	France	Gard	Gard	43.945	4.151	-
H_FR_GAR_gj01	Alves et al 2019	1908	France	Gard	Gajan	43.897	4.215	-
H_FR_GAR_gj02	Alves et al 2019	1908	France	Gard	Gajan	43.897	4.215	-
H_FR_GAR_po01	Alves et al 2019	1908	France	Gard	Poulx	43.911	4.424	-
H_FR_GAR_po02	Alves et al 2019	1908	France	Gard	Poulx	43.911	4.424	-
H_FR_GAR_po03	Alves et al 2019	1908	France	Gard	Poulx	43.911	4.424	-
H_FR_GAR_sd01	Alves et al 2019	1932	France	Gard	St Géniès de Malgoirès	43.946	4.214	-
H_FR_GAR_sg01	Alves et al 2019	1908	France	Gard	St Gilles	43.677	4.434	-
H_FR_LOI_ne01	Alves et al 2019	1943	France	Loir et Cher	Neuvy	47.563	1.603	-
H_FR_LOI_ne02	Alves et al 2019	1943	France	Loir et Cher	Neuvy	47.563	1.603	-
H_FR_RHO_ec01	Alves et al 2019	1900	France	Rhône	Ecully	45.775	4.779	-
H_FR_SAO_sv01	Alves et al 2019	1908	France	Saône-et-Loire	Saint-Verand	45.905	4.508	-
M_NZ_BOP_tp01	New data	2013	New Zealand	Bay of Plenty	Te Puke	-37.830	176.330	-
M_NZ_CAN_sh01	New data	2012	New Zealand	Canterbury	Simons Hill	-44.198	170.299	-
M_NZ_MAR_mo01	New data	2013	New Zealand	Marlborough	Molesworth	-42.159	173.092	-
M_NZ_SOU_ws01	New data	2013	New Zealand	Southland	Wantwood Station Gore	-45.990	168.765	-
M_NZ_WEL_ov01	New data	2012	New Zealand	Wellington	Orongorongo Valley	-41.396	174.918	-
M_TS_TAS_lf01	New data	2012	Tasmania	Tasmania	Longford	-41.560	147.119	-
M_TS_TAS_sf01	New data	2017	Tasmania	Tasmania	Sandford	-42.928	147.524	-
M_UK_BUK_hw01	Alves et al 2019	2012	United Kingdom	Buckinghamshire	High Wycombe	51.635	-0.810	-
M_UK_CAM_md01	Alves et al 2019	2012	United Kingdom	Cambridgeshire	Madingley	52.221	0.046	-
M_UK_CAM_md02	Alves et al 2019	2012	United Kingdom	Cambridgeshire	Madingley	52.228	0.043	-
M_UK_CAM_pt01	Alves et al 2019	2012	United Kingdom	Cambridgeshire	Peterborough	52.542	-0.325	-
M_UK_CAM_pt02	Alves et al 2019	2012	United Kingdom	Cambridgeshire	Peterborough	52.540	-0.323	-
M_UK_DEV_lt01	Alves et al 2019	2012	United Kingdom	Devon	Lettaford	50.641	-3.837	-
M_UK_DEV_lt02	Alves et al 2019	2012	United Kingdom	Devon	Lettaford	50.641	-3.837	-
M_UK_ESS_lg01	Alves et al 2019	2012	United Kingdom	Essex	Langham	51.943	0.935	-
M_UK_ESU_pp01	Alves et al 2019	2012	United Kingdom	East Sussex	Plumpton	50.892	-0.042	-
M_UK_ESU_pp02	Alves et al 2019	2012	United Kingdom	East Sussex	Plumpton	50.892	-0.042	-
M_UK_HAM_ss01	Alves et al 2019	2012	United Kingdom	Hampshire	Sutton Scotney	51.154	-1.338	-

M_UK_HAM_ss02	Alves et al 2019	2012	United Kingdom	Hampshire	Sutton Scotney	51.154	-1.338	-
M_UK_KEN_gd01	Alves et al 2019	2012	United Kingdom	Kent	Goodnestone	51.319	0.930	-
M_UK_KEN_gd02	Alves et al 2019	2012	United Kingdom	Kent	Goodnestone	51.319	0.930	-
M_UK_KEN_hr01	Alves et al 2019	2012	United Kingdom	Kent	Harrietsham	51.247	0.679	-
M_UK_NTP_ha01	Alves et al 2019	2012	United Kingdom	Northamptonshire	Harlestone	52.281	-0.982	-
M_UK_NTP_pd01	Alves et al 2019	2012	United Kingdom	Northamptonshire	Preston Deanery	52.186	-0.845	-
M_UK_SRP_sh01	Alves et al 2019	2012	United Kingdom	Shropshire	Shrewsbury	52.663	-2.678	-
M_UK_STA_we01	Alves et al 2019	2012	United Kingdom	Staffordshire	Weston Park, Tong	52.667	-2.303	-
M_UK_SUF_ww01	Alves et al 2019	2012	United Kingdom	Suffolk	Walsham le Willows	52.303	0.920	-
M_UK_SUF_ww02	Alves et al 2019	2012	United Kingdom	Suffolk	Walsham le Willows	52.303	0.920	-
M_UK_SUR_cp01	Alves et al 2019	2012	United Kingdom	Surrey	Capel	51.151	-0.312	-
M_UK_SUR_cp02	Alves et al 2019	2012	United Kingdom	Surrey	Capel	51.115	-0.324	-
M_UK_WSU_pg01	Alves et al 2019	2012	United Kingdom	West Sussex	Partridge Green	50.963	-0.296	-
M_UK_WSU_pg02	Alves et al 2019	2012	United Kingdom	West Sussex	Partridge Green	50.963	-0.296	-
H_UK_BUK_ml01	Alves et al 2019	1912	United Kingdom	Buckinghamshire	Marlow	51.575	-0.780	-
H_UK_BUK_ml02	Alves et al 2019	1912	United Kingdom	Buckinghamshire	Marlow	51.575	-0.780	-
H_UK_CAM_as01	Alves et al 2019	1954	United Kingdom	Cambridgeshire	Peterborough, Ashton Wold	52.637	-0.374	-
H_UK_CAM_as02	Alves et al 2019	1954	United Kingdom	Cambridgeshire	Peterborough, Ashton Wold	52.637	-0.374	-
H_UK_DEV_ht01	Alves et al 2019	1937	United Kingdom	Devon	Hatherleigh	50.819	-4.072	-
H_UK_DEV_kb01	Alves et al 2019	1876	United Kingdom	Devon	Near Kingsbridge	50.284	-3.777	-
H_UK_DOR_se01	Alves et al 2019	1934	United Kingdom	Dorset	Seatown, Bridport	50.722	-2.824	-
H_UK_DOR_se03	Alves et al 2019	1893	United Kingdom	Dorset	Bridport, Near Seatown	50.722	-2.824	-
H_UK_ESU_dt01	Alves et al 2019	1908	United Kingdom	East Sussex	Ditchling	50.918	-0.116	-
H_UK_GLA_pe01	Alves et al 2019	1914	United Kingdom	Glamorgan	Pendolyan	51.482	-3.356	-
H_UK_HAM_an01	Alves et al 2019	1944	United Kingdom	Hampshire	Andover	51.211	-1.492	-
H_UK_HAM_ev01	Alves et al 2019	1894	United Kingdom	Hampshire	Eversley	51.352	-0.889	-
H_UK_HAM_em01	Alves et al 2019	1928	United Kingdom	Hampshire	Emsworth	50.848	-0.938	
H_UK_HER_bk01	Alves et al 2019	1903	United Kingdom	Hertforshire	Berkhamsptead	51.760	-0.568	-
H_UK_KEN_ke02	Alves et al 2019	1869	United Kingdom	Kent	Kent	51.279	0.522	-
H_UK_KEN_pl01	Alves et al 2019	1912	United Kingdom	Kent	Pluckley, Surrenden Park	51.174	0.771	-
H_UK_KEN_pl02	Alves et al 2019	1912	United Kingdom	Kent	Pluckley, Surrenden Park	51.174	0.771	-
H_UK_KEN_pl03	Alves et al 2019	1912	United Kingdom	Kent	Pluckley, Surrenden Park	51.174	0.771	-
H_UK_NTP_nt01	Alves et al 2019	1882	United Kingdom	Northamptonshire	-	52.273	-0.876	-

H_UK_NTP_sp01	Alves et al 2019	1945	United Kingdom	Northamptonshire	Spanhoe	52.568	-0.633	-
H_UK_NTP_sp02	Alves et al 2019	1945	United Kingdom	Northamptonshire	Spanhoe	52.568	-0.633	-
H_UK_SOM_cv01	Alves et al 2019	1924	United Kingdom	Somerset	Clevedon	51.442	-2.856	-
H_UK_SOM_cv02	Alves et al 2019	1924	United Kingdom	Somerset	Cleredon	51.442	-2.856	-
H_UK_STA_ch01	Alves et al 2019	1925	United Kingdom	Staffordshire	Chartley	52.852	-1.988	-
H_UK_STA_ch02	Alves et al 2019	1925	United Kingdom	Staffordshire	Chartley	52.852	-1.988	-
H_UK_STA_ch03	Alves et al 2019	1925	United Kingdom	Staffordshire	Chartley	52.852	-1.988	-
H_UK_STA_ut01	Alves et al 2019	1925	United Kingdom	Staffordshire	Uttoxeter	52.898	-1.866	-
H_UK_SUR_cy01	Alves et al 2019	1898	United Kingdom	Surrey	Croydon	51.357	-0.098	-
H_UK_SUR_nd01	Alves et al 2019	1934	United Kingdom	Surrey	Newdigate, Oaklands Park	51.143	-0.259	-
H_UK_WSU_cw01	Alves et al 2019	1865	United Kingdom	West Sussex	Cowfold	50.991	-0.273	-

Table S2 - Summary of the analyses of polymorphism and frequency spectrum tests of neutrality in the protein-coding sequence (CDS) variants. N corresponds to the number of individuals used in the analysis. Nucleotide diversity is measured as the average number of pairwise differences in a sample (70). Historical samples were excluded from the analysis.

Population	**n**	**Tajima's *D***	**Genetic Diversity**
France	26	0.203	0.1091%
Britain	25	0.888	0.0975%
Australia	40	1.123	0.0855%
New Zealand	5	0.462	0.0737%
Domestic	8	0.616	0.0701%
Tasmania	2	0.185	0.0574%
Australia (Victoria/NSW)	7	0.716	0.0803%
Australia (Queensland)	7	0.736	0.0770%
Australia (South Australia)	7	0.604	0.0717%
Australia (Western Australia)	7	0.732	0.0703%
Australia (Sydney)	4	0.379	0.0615%
Australia (Cattai)	1	-	0.0414%