

Are Sydney rabbits different?

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ABSTRACT

Rabbits have been present in the Sydney district since well before the Geelong release that provided the genetic stock of rabbits seen throughout most of Australia. In this study a comparison was made between the genetic variation present in, and the endoparasitic communities of, rabbits in the Sydney region and elsewhere in Australia. A genetic variant in the mtDNA control region is common in the Sydney population studied but is not found elsewhere in Australia. The allozyme variants present are similar to those found in inland NSW, though a rare phosphogluconate dehydrogenase allele is missing from the three high rainfall populations (Sydney, Mogo and Bemboka) studied. While different coat colours are found in rabbits in inland NSW, 'blue' rabbits are not found in Sydney rabbit populations and 'ginger' rabbits are very rare. The suite of ten nematode and protozoan parasite species present in Sydney populations is the same as that found elsewhere in NSW. Six parasitic species found in England are not found in Australia. However, they are also not found in New Zealand, one of the possible alternate sources of Sydney rabbits. The biology of the parasites in the Sydney region is similar to that elsewhere in Australia. It seems likely that rabbit populations in the Sydney area maintain genetic variation derived from both the Geelong release and at least one local release near Sydney. The divergences in the gene pool are minor and it is likely that the rabbits will not show any significant differences in their ecology from those elsewhere, a result supported by the similarity in the ecology of the parasite suite.

Key Words: rabbits, *Oryctolagus*, mtDNA, allozymes, parasitology, *Eimeria*

Introduction

Rabbits have been present in Australia since the arrival of the First Fleet (Stodart and Parer 1988; Myers *et al.* 1994). Five rabbits accompanied the British into Port Jackson (Sydney), and rabbits were reported to be breeding around the settlement in 1825 (Stodart and Parer 1988). Over the next 30 years, many attempts were made to introduce the rabbit to various locations in Australia by the acclimatisation movement (Rolls 1969). These attempts were mostly made using semi-domestic breeds, which were unsuitable for survival in the wild, and many colonies failed to establish. A population based on six English wild rabbits and seven colour-morphic semi-domesticated rabbits was successfully established

at Barwon Park, near Geelong in Victoria in 1859 ('Geelong Release'; Rolls 1969; Palmer 1993). These rabbits, together with those from a possibly derivative colony at Kapunda, near Adelaide, are thought to be the main sources from which rabbits spread across the southern two-thirds of mainland Australia. While the rate of spread was rapid in inland Australia, the rabbit only slowly moved into the higher rainfall areas of eastern New South Wales (Stodart and Parer 1988).

In 1875, before rabbits from the expanding populations in Victoria and South Australia are documented to have spread to N.S.W., rabbits were recorded to be thoroughly established around Sydney (Stodart and Parer 1988). In 1860 a colony was established on a property owned by

Mr Thomas Holt on the Cooks River near Sydney. The sixty rabbits used to establish this colony were imported from Tasmania and New Zealand and by 1864 the population on the property consisted of 2-3000 rabbits (Stodart and Parer 1988). The question can be asked then as to the nature and attributes of rabbits in the Sydney district compared to those in other parts of Australia given they may be independently derived from Europe or show attributes derived from more than one release.

There are many methods of determining whether sub-populations of a species found at different geographical locations belong to different breeding stocks. Two methods are commonly used in such analyses. Firstly, the analysis of the geographical distribution of genetic variation can be examined and significant divergences may be found, as for example rabbits in south and central Tasmania compared to northern Tasmania (Richardson *et al.* 1980). Secondly, differences in the suites of parasites present may be found in different areas. The latter method is more commonly used in fisheries studies than in studies of terrestrial species (MacKenzie and Abaunzab 1998).

The objectives of this study were to describe the range of genetic variation found in rabbits in the Hawkesbury district near Sydney and to compare these results with those found for rabbit populations in inland eastern Australia. A further purpose was to determine whether the suite and patterns of endoparasitic infection observed here were different to those found in rabbits in other parts of Australia. The nature and significance of any differences in genetic profile and parasite ecology are then considered.

Methods

Genetics

The genetic comparisons made were based on allozyme variation detected using electrophoresis and on DNA sequence differences in the control region of the mitochondrial DNA (mtDNA). In each case material from the Hawkesbury District was typed and the results compared with those obtained in studies of other populations.

Blood samples (approx. 1-1.5mL) were obtained via cardiac extraction from live-trapped rabbits at Hope Farm in Cattai National Park 50km northeast of Sydney (33.4°S, 150.9°E). Live trapping was carried out, using freshly chopped carrot as bait, for four consecutive nights at

fortnightly intervals over a period of four years. Trapped rabbits were tagged using numbered plastic tags (Dalton kitten tags) and the weight and sex of the rabbit determined. The blood was stored on ice in tubes containing anticoagulant (potassium oxalate and sodium fluoride). Upon returning from the field, the blood was centrifuged and the plasma removed. The blood cells were mixed with a cryopreservative (equal volume of 6% sodium citrate in 40% ethylene glycol) and stored at -20°C.

Mitochondrial DNA sequences were obtained from purified DNA prepared from the blood samples. Preserved blood cells (50µL) were placed in a 1.5mL microcentrifuge tube with 250 µL of digestion buffer (10mM Tris-HCL pH 8.0, 10mM EDTA, 50 mM NaCl and 2% SDS) and 10µL of proteinase K solution (10 mg/mL). After vortexing, the mixture was incubated at 56°C for one hour. Following centrifugation and separation of the supernatant, any remaining impurities were removed by phenol chloroform extractions and ethanol precipitation. The concentration and purity of each DNA sample was then checked and the sample stored at -20°C until used for PCR amplification.

Mitochondrial control region DNA was amplified by polymerase chain reaction (PCR) using specific primers that were designed to amplify a 667 bp region from the tRNA-proline gene through to the end of the central conserved region left of CSB1: the light strand primer (d11 588), 5'-AGGCTCCTGCCCCACCAGC-3' and the heavy strand primer (dlr 1254), 5'-ACATCCACAGTTATGTGTGAGC-3'. PCR amplifications were performed in 125µL reaction volumes using 100ng DNA, 10mM Tris-HCL (pH 9.0), 50mM KCL, 3.1mM MgCl₂, 320 µM of each dNTPs, 2.5 U *Taq* DNA Polymerase (Promega), 120nM of both primers (one phosphorylated) and 60µL of mineral oil. The cycling conditions were as follows: Step (1) 94°C for 2 min, Step (2) 94°C for 30 sec, 65°C for 2 min, 72°C for 3 min (25 cycles), Step (3) 72°C for 5 min, Step (4) stop at 5°C. Purified ssDNA was prepared by digestion with λ exonuclease enzyme (Higuchi and Ochman 1989), followed by phenol-chloroform extraction and ethanol precipitation. Single stranded sequencing was performed on both mtDNA strands using a T7 Sequencing kit (Pharmacia) and resolved on 8% polyacrylamide gels which were exposed to X-ray film (Fuji) overnight. The sequences were then compared, with haplotype identity and

frequencies compared with data from five other Australian populations (Zenger 1996). To eliminate the possibility of *Taq* errors among the closely related haplotypes, sequencing was repeated for key animals.

The samples were typed for previously reported electrophoretically detectable allozyme variation on cellulose acetate ('Cellogel') gels using the general methods described in Richardson *et al.* (1986). The loci studied were adenosine deaminase (E.C. 3.5.4.4) (2 alleles), dihydrolipamide reductase (NAD⁺) (diaphorase) (3 alleles) (E.C. 1.6.4.3) and phosphogluconate dehydrogenase (E.C. 1.1.1.44) (2 alleles). The specific running conditions used are given in Richardson *et al.* (1980). Allele frequencies for other Australian populations were obtained from Richardson (1980).

Parasitology

The rabbit parasitology study site was located on the Hawkesbury campus of the University of Western Sydney, Hawkesbury (33.62°S, 150.75°E), approximately 50 kilometres north west of Sydney. The field capture methods were the same as those used at the Cattai site, and were carried out over a period of eight months. Sixty treadle or bar operated traps were used to sample the population of 24 warrens. Fresh faecal pellets were collected from the ground underneath each occupied trap.

Faecal oocyst/egg counts were used as an indication of the parasite burdens of the population. These were undertaken on material collected using a salt floatation technique in saturated sodium chloride (Dow 1962). Counts were transformed to $\log_{10}(x+1)$ to give a geometric mean for the samples.

Nematode eggs and protozoan oocysts were separated on the basis of size, shape, internal structure and colour. *Eimeria* oocysts were further identified, following Levine and Ivens (1972), on the basis of size, shape of the entire oocyst, shape of the sporocysts and the presence or absence of a residual body. Oocysts belonging to *E. media* and *E. stiedae* were not always able to be confidently separated and therefore were grouped together for analysis. The species of nematode eggs were differentiated primarily on the size and shape of the egg, according to measurements given by Bull (1953) and Soulsby (1982).

Results obtained for all male adult rabbits were grouped together, as were counts obtained from female adult rabbits. Adults were defined as rabbits weighing over 1200g at the time of capture. Young rabbits were only present in the population in sufficiently high numbers from June through to September; therefore comparisons between adults and kittens (400-800g) were only made for this period. Differences in the intensity of endoparasite infection between male and female, and adult and juvenile rabbits, were analysed using one way ANOVAs. Differences in the prevalence of endoparasite infection were analysed using the G test.

Results

Genetics

The mtDNA haplotype frequency data (Table 1) show the Cattai population to be markedly different from any of the other populations studied in Australia. A haplotype (Aus-7) is found at high frequency (0.5) that has not been found in any other population. The remaining three haplotypes located here are the most common haplotypes shared across the majority of the other populations. Pairwise nucleotide comparisons of Aus-7 (Genbank accession number AF003195) to the other six other haplotypes found in Australia (accession numbers AF003189 to AF003194) revealed a minimum of 5 and a maximum of 13 nucleotide differences to Aus-1 and Aus-2 respectively, which translates to 0.885 and 2.301 percent nucleotide divergence. A further haplotype (Aus-3) may be present at Cattai, however haplotypes Aus-3 and Aus-6 differ by a single base change in the central section of the control region, a site far removed from the remaining variant positions in the left region. Not all animals from Cattai were typed for the central section and consequently both Aus-3 and Aus-6 haplotypes may be present, however all those typed for the central section were Aus-6.

Examination of the results for allozyme allele frequencies given in Table 2 shows that there was little difference between Cattai and the previously studied Geelong release populations for either adenosine deaminase or diaphorase. Examination of the phosphogluconate dehydrogenase data shows that the rare allele is missing from the Cattai and Mogo populations.

Table 1. The frequencies of haplotypes for the mitochondrial control region collected for the rabbit population from Cattai and data for other eastern Australian sites taken from Zenger (1996). * Aus-6 may include animals with haplotype Aus-3 (see text).

Location	mtDNA Haplotype Frequency						n
	Aus-1	Aus-2	Aus-4	Aus-5	Aus-6*	Aus-7	
Cattai NSW	0.22	0.07	0	0	0.20	0.50	54
Colac Vic	0.61	0.20	0.06	0.02	0.10	0	49
Hillston NSW	0.08	0.08	0	0	0.84	0	49
Bourke NSW	0.28	0.19	0	0	0.53	0	47

Table 2. The allele frequencies for three allozyme loci collected for the rabbit population at Cattai and the equivalent data reported in Richardson et al. (1980) for other locations in N.S.W..

Location	Locus									
	Ada			Dia			Pgd			
	1	2	3	2n	F	S	2n	1	2	2n
Cattai NSW	0.59	0.40	0.11	308	0.74	0.26	422	1	0	100
Grassy Creek ACT	0.67	0.29	0.04	382	0.75	0.25	130	0.98	0.02	396
Urana NSW	0.54	0.43	0.03	730	0.76	0.24	108	0.93	0.07	788
Mogo NSW	0.56	0.42	0.02	60	-	-	-	1	0	58

Variously coloured rabbits ('ginger', 'black' and 'blue' (or 'smoky')) occur in populations derived from the Geelong release at frequencies ranging between 0.4-3.8%, 0-0.6%, 0-1.5%, respectively (Stodardt 1965). No 'blue' rabbits have been collected in the Sydney area in the many tens of thousands of poisoned rabbits seen by the Rural Lands Board, nor have they been seen by the authors. A few isolated examples of ginger rabbits have been seen by the Rural Lands Board staff. 'Black' rabbits are seen more frequently, reaching locally an estimated 2% in some wetter, coastal, regions (A. Glover, Rural Lands Board, pers. com.).

Parasitology

Ten species of endoparasites were present in the faecal samples of the rabbit population at Richmond. These consisted of three nematode species and seven protozoan species (Table 3). This is the same suite of parasites found in other parts of eastern Australia and in New Zealand. Six other endoparasites *Eimeria intestinalis*, *E. flavescens*, *Cittotaenia pectinata*, *C. denticulata*, *C. ctenoides* and *Andrya cuniculi* are found in English rabbit populations (Dunsmore, 1981) but not reported in either Australia or New Zealand.

Table 2. The allele frequencies for three allozyme loci collected for the rabbit population at Cattai and the equivalent data reported in Richardson et al. (1980) for other locations in N.S.W..

Species	Infection rate (%)			Infection Intensity (log(x+1)±S.E.)		
	Adult Male	Adult Female	Kittens	Adult Male	Adult Female	Kittens
<i>Eimeria media/stiedae</i>	96	87	43	3.44±0.16	2.90±0.17	3.82±0.22
<i>Eimeria perforans</i>	88	87	43	3.18±0.20	2.96±0.19	4.35±0.28
<i>Eimeria exigua</i>	43	37	13	0.92±0.16	0.81±0.14	2.13±0.23
<i>Eimeria piriformis</i>	61	51	30	2.21±0.27	1.75±0.23	3.35±0.32
<i>Eimeria magna</i>	20	14	52	0.57±0.13	0.43±0.14	3.97±0.25
<i>Eimeria irresidua</i>	0	3	0	0	0.09±0.06	0
<i>Trichostrongylus retortaeformis</i>	41	29	17	0.96±0.18	0.63±0.13	2.63±0.32
<i>Passalurus ambiguus</i>	8	3	9	0.24±0.12	0.07±0.05	2.73±0.03
<i>Graphidium strigosum</i>	4	3	0	0.09±0.06	0.05±0.04	0
Sample size	49	63	23	49	63	23

The data on the prevalence and intensity of parasitic infections are summarised in Table 3. Differences in the prevalence of protozoan and nematode infections between male and female rabbits were not detected. The intensity of infection of *E. magna* was found to be significantly higher in female rabbits than male rabbits ($df=16$, $F=5.02$, $p<0.05$). This was the only species of protozoan or nematode for which a difference in the intensity of infection between male and female rabbits was detected. Significant differences in the prevalence of endoparasite infection between adult and juvenile rabbits occurred for some of the protozoan species. The prevalence of *E. perforans* ($df=1$, $G=15.8$, $p<0.001$), *E. piriformis* ($df=1$, $G=14.1$, $p<0.001$) and *E. media/E. stiedae* ($df=1$, $G=4.01$, $p<0.05$) infections were higher in adult rabbits than juvenile rabbits. The prevalence of *E. magna* infections was higher in juvenile rabbits ($df=1$, $G=20.1$, $p<0.001$). The eggs of *G. strigosum* were only found to be present in the faecal samples of adult rabbits. *Eimeria irrisidua* oocysts were not found in faecal samples from either adult or juvenile rabbits in spring. The intensity of infections were significantly higher in juvenile rabbits than adult rabbits for *E. media/E. stiedae* ($df=1$, $F=4.97$, $p<0.05$), *E. perforans* ($df=1$, $F=7.22$, $p<0.01$) and *E. magna* ($df=1$, $F=10.1$, $p<0.001$) (Table 3). *Trichostrongylus retortaeformis* infections appeared to be more prevalent in adult rabbits, however, the difference observed was not significant.

Discussion

The identification of the area of origin of the population in Sydney based on the mtDNA data is uncertain. While six haplotypes are described from five populations studied in other parts of Australia (Zenger 1996), the Cattai population contains only the subset Aus-1, Aus-2 and Aus-6 (plus possibly Aus-3). These are, however, the most common haplotypes found elsewhere in Australia. The Cattai population also includes a unique allele at high frequency not found elsewhere. The high frequency of Aus-7 and the relatively large sequence divergence between it and the other haplotypes excludes the possibility of this haplotype originating via mutation in Australia, given the population history and the time available since arrival. This result would support the hypothesis of a contribution to the gene pool from at least one separate release in the Sydney region. It does not preclude of course the

possibility of a contribution of genetic variation from the Geelong release. The Geelong release also included coloured hutch rabbits (Palmer 1993) and it is not uncommon to find 'ginger', 'blue' and 'black' animals in various parts of inland Australia (Stodardt 1965). Black rabbits are extremely common (20-30%) in some parts of Tasmania (Barber 1954), one of the possible sources of the rabbits released at Cooks River near Sydney. The absence of 'blue' rabbits and the extreme rarity of 'ginger' rabbits support the mtDNA data as evidence for a significant contribution to the local gene pool from sources other than Geelong. The presence of 'gingers' may be taken as evidence of some contribution of genes from Geelong release rabbits to the population, but whether this is by natural gene flow or by the release of the occasional 'ginger' rabbit collected elsewhere as a curiosity is uncertain. It is also clear that rabbit populations in closely settled areas, for example, Sydney's Centennial Park, include large genetic contributions from released pet rabbits (as reflected in size, form and colour, authors unpubl. data). Coloured rabbits are also not seen in wild rabbit populations in England (D. Bell pers. com.).

The allozyme data show results similar to those for other NSW populations for all loci except phosphogluconate dehydrogenase. While the rare allele is absent from the Cattai population, its distribution elsewhere in NSW is patchy. In previous studies (Richardson *et al.* 1980) it was shown to be present in seven populations in inland NSW but missing from Mogo and Bemboka in higher rainfall areas. It seems unlikely that Sydney release rabbits could have spread as far as Bemboka and the differences may be explained in terms of genetic drift or selection (Richardson *et al.* 1980). Both alleles are present in Europe and also, along with a third allele, on Macquarie Island (Richardson unpubl.).

The 10 species of endoparasites identified in the faecal samples of rabbits from this study were amongst the 13 species recognised as the major species of endoparasites of the rabbit in Australia (Myers *et al.* 1994). The remaining three species are Platyhelminthes which utilise the rabbit as an intermediate host and are not found in the faeces of the rabbit. The species of endoparasites observed in the faeces of rabbits from this study are the same suite of species identified in the rabbit in New Zealand (Bull 1953). As a consequence, it is not possible to discriminate between the possible origins of the

Sydney rabbits on the basis of parasites, as two of the potential source populations carry the same set of species. The six extra species found in Britain but not found in Australasia were presumably lost on each occasion during the long sea voyage to the antipodes.

The lack of difference between the intensity of endoparasite infections in male and female rabbits in this study (Table 3) is similar to the pattern observed by Dunsmore (1966a, b, c) who studied *T. retortaeformis*, *P. ambiguus* and *G. strigosum* in four different climatic regions across eastern Australia. *Graphidium strigosum* eggs were only found in the faeces of adult rabbits. The absence of the eggs of this species in younger rabbits is supported by previous studies (Bull 1964, Dunsmore 1966b). The intensity of *E. media*/*E. stiedae*, *E. perforans* and *E. magna* infections (Table 3) were significantly higher in juvenile rabbits than in adults. Stodart (1968) and Mykytowycz (1962) observed that decreasing oocyst levels were

generally associated with increasing age of the rabbits in other areas of Australia

The differences in the suites of mitochondrial haplotypes, and perhaps the reduced presence of coloured rabbits, would support the view that rabbit populations in the Sydney area have a mixed origin and include a genetic contribution not found in rabbits elsewhere in Australia. The presence of the same suite of parasites and the similarities in the allozyme profiles are ambiguous and do not provide support for any hypothesis regarding origins. The allozyme differences between the Geelong release and Sydney rabbits are far less than that found between populations in different parts of Tasmania (Richardson et al. 1980). As a consequence it is unlikely that any ecologically significant, genetically-based, differences would be found between the rabbits in Sydney and those elsewhere in Australia and such a result is reflected in the parasite epidemiological data.

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