Low major histocompatibility complex diversity in the Tasmanian devil predates European settlement and may explain susceptibility to disease epidemics

Katrina Morris¹, Jeremy J. Austin²,³ and Katherine Belov¹

¹Faculty of Veterinary Science, University of Sydney, RMC Gunn B19, Sydney, New South Wales 2006, Australia
²Australian Centre for Ancient DNA, School of Earth and Environmental Sciences, The University of Adelaide, Adelaide, South Australia 5005, Australia
³Sciences Department, Museum Victoria, Carlton Gardens, Melbourne, Victoria 3000, Australia

The Tasmanian devil (Sarcophilus harrisii) is at risk of extinction owing to the emergence of a contagious cancer known as devil facial tumour disease (DFTD). The emergence and spread of DFTD has been linked to low genetic diversity in the major histocompatibility complex (MHC). We examined MHC diversity in historical and ancient devils to determine whether loss of diversity is recent or predates European settlement in Australia. Our results reveal no additional diversity in historical Tasmanian samples. Mainland devils had common modern variants plus six new variants that are highly similar to existing alleles. We conclude that low MHC diversity has been a feature of devil populations since at least the Mid-Holocene and could explain their tumultuous history of population crashes.

1. Introduction

The Tasmanian devil (Sarcophilus harrisii) is the world’s largest remaining carnivorous marsupial. Devils were once widespread on the Australian mainland [1], but the species is now restricted to the island state of Tasmania that has been separated from the Australian mainland for 10,000 years. Humans first arrived in Australia around 50,000–65,000 years ago [2]. A small number of wild dogs (dingoes) were introduced from South East Asia 5000–10,000 years ago [3]. They spread across mainland Australia but never reached Tasmania. By 10,000 years ago, the mainland devil population was reduced to three relict populations in the north, southwest and east of Australia [4], and extinction on the mainland occurred about 3000–4000 years ago [4]. Extinction on the mainland is believed to be due to competition from dingoes [5] although climate change [4], increased hunting by the expanding human population [6] and the introduction of a pathogen from dingoes [5] have also been suggested to be contributing factors.

Historical and anecdotal records indicate that there have been several declines in the devil population in Tasmania since the arrival of Europeans in 1804 [7]. The cause of these population declines is unknown, however, anecdotal reports of a ‘distemper-like’ disease affecting large dasyurids coincided with a substantial drop in devil sightings early in the 1900s [7–9].

Population declines in devils may be the cause of, or the result of, restricted genetic diversity. Jones et al. [10] found that devils have low diversity at microsatellite markers, whereas Miller et al. [11] found low diversity in the mitochondrial genome of 14 individuals and SNP markers in 175 individuals. Most significantly, low diversity is present in the major histocompatibility complex (MHC) genes in devils [12,13]. MHC proteins are present on the surface of most cells, and are critical in immune recognition of pathogens and foreign or
infected cells. Devil class I sequences show 88.5 per cent average nucleotide identity [14] compared with grey short-tailed opossum (*Monodelphis domestica*) sequences that exhibit between 49 and 83 per cent identity [15]. The lack of MHC diversity in Tasmanian devils may have facilitated the spread of the most recent disease epidemic, the devil facial tumour disease (DFTD) [12]. This disease has led to a population decline of 84 per cent, and extinction in the wild is likely within 35 years, unless the spread of the disease can be stopped [16,17].

Why devils have such low MHC diversity is unknown. It is possible that one or more of the population declines since the arrival of Europeans in Tasmania resulted in depleted MHC diversity, or that MHC diversity was already limited in Tasmania. Alternatively, low MHC diversity may predate the separation of Tasmania from the mainland. In order to investigate this question, we cloned and sequenced class I MHC alleles in historical devil samples. Only two studies have previously sequenced MHC alleles in historical samples. Eimes *et al.* [18] studied MHC in specimens dating to 1951–1952 in prairie chickens, whereas Smulders *et al.* [19] studied class II MHC alleles in common hamster (*Cricetus cricetus*) specimens dating back to 1924.

### 2. Material and methods

Full methods, including precautions taken against contamination with modern DNA, are detailed in the electronic supplementary material. In brief, small bone or tissue samples were taken from museum specimens of mainland and Tasmanian devils. Sample locations are shown in figure 1 and additional sample details are in the electronic supplementary material, table S1. DNA was extracted from 100 mg bone powder or 3 mm³ of dried tissue using a modified QIAGEN DNeasy Blood and Tissue kit. MHC
class I alleles were amplified using PCR. Products were separated by gel electrophoresis and purified using the QIAquick Gel Extraction kit (QIAGEN). Samples were cloned using the pGEM-T Easy vector system (Promega, Madison, WI, USA) and sequenced.

3. Results

Fifty alleles were amplified, cloned and sequenced from 14 individuals (see the electronic supplementary material, figure S1). Full αI domains were characterized from devils from 1922 to 1987. Between four and five alleles were amplified from each individual, and MHC complements were very typical of present day MHC complements. The four most common sequence variants from modern devils make up 59 per cent of the variants present in the 1922–1987 samples, compared with 58 per cent in previously studied modern devils [20]. The frequency of alleles in the 1922–1987 samples were comparable to those from modern devils (see the electronic supplementary material, figure S2), with no significant difference in the frequency of alleles in modern and historical samples ($\chi^2$ test, $p = 0.69$). No new alleles were seen in this population.

For the samples dating to the 1800s or earlier, shorter fragments targeting the peptide binding region (PBR) of the αI domain were amplified. Two of these were samples collected in Tasmania after European colonization, whereas five samples date to before European colonization. Between one and four variants were amplified in these samples. It is likely that variants have been missed in some of these samples, owing to DNA degradation and low concentration, in particular with the sample A-WA, where only one variant was amplified. The ancient Tasmanian variants match common modern alleles across the PBR with no novel amino acid substitutions in this region. This includes the Tasmanian sample predating European colonization.

In mainland Australian animals, two variants were identified that matched to common modern variants in the PBR. One of these (SahaI*pa4) was present only in a single Victorian sample, whereas the other variant (SahaI*pb1) was present in two Victorian samples and in one Western Australian sample. The remaining variants identified in the mainland samples (SahaI*pa5-10) were new alleles. There were four novel amino acid substitutions in these new variants. All of these substitutions occurred at positions involved in peptide binding. The new variants are closely related to modern sequences (figure 2); variants SahaI*pa6-9 cluster with modern sequences, whereas SahaI*pa10 is more divergent.

Figure 2. Statistical parsimony phylogenetic network for 140 MHC alpha I sequences from contemporary (greater than 1900, white), historic (1800 – 1900, horizontal lines) and ancient (Late Pleistocene – Mid-Holocene, grey) Tasmanian devils and ancient (Late Pleistocene – Mid-Holocene, black) mainland devils. Numbers refer to allele numbers and small black circles represent unobserved alleles.
4. Discussion

We sampled MHC diversity at four critical time periods in devil evolutionary history—Late Pleistocene to Mid-Holocene mainland Australia, Late Pleistocene to Mid-Holocene Tasmania, nineteenth century Tasmania (immediately after European colonization) and mid-twentieth century Tasmania (but prior to DFTD). Unlike previous temporal studies of ‘neutral’ genetic diversity in extinct and endangered species, we have focused on functional genes and report the oldest characterized MHC alleles, by at least 3000 years [19].

All the Tasmanian samples sequenced in this study, including the sample that predates European colonization, showed an MHC complement that is typical of modern-day devils. Based on these results it is probable that low MHC diversity has persisted for many hundreds of years in Tasmania. It is interesting to note that low genetic diversity was recently observed in ancient remains of the thylacines from Tasmania [21].

Low MHC diversity is also present in extinct mainland devils. We postulate that prior to the separation of Tasmania from the mainland, devils had low genetic diversity, following population contractions during the Pleistocene and Holocene [4]. Low MHC diversity would have impeded the ability of devil populations to resist disease outbreaks and may have contributed to the extinction of devils on the mainland. It is possible that dingoes may have brought with them new diseases, such as distemper, previously unencountered by Australian wildlife. Disease along with competition with dingoes may have been the final straw for devils on the mainland. Devils persisted in Tasmania in the absence of dingoes. However, the introduction of dogs to Tasmania in the early 1800s may have been the cause of the disease epidemics that affected Tasmanian devils in the nineteenth and twentieth centuries.

A distemper-like disease [7] in both Tasmanian devils and thylacines in the early twentieth century [9] may have led to a population crash in eastern devil populations [13]. Distemper in Australian dogs is rare, but outbreaks do occur [22]. Such a disease may have further reduced MHC diversity via a selective sweep, leading to low diversity at the MHC and higher diversity genome-wide, including at minor histocompatibility antigens. This scenario may help explain why skin grafts in MHC similar devils were rejected [23]. To confirm the presence of distemper in eastern devil population, it may be possible to examine museum specimens for evidence of canine distemper.

In summary, we have shown that low levels of MHC diversity have been a characteristic of Tasmanian devil populations for hundreds, if not thousands, of years and that low genetic diversity in present day populations is not due to recent human impacts. Tasmanian devils provide a stark reminder that maintenance of genetic diversity at genes involved in immunity is critical for long-term survival of species. Careful monitoring of populations with low diversity is necessary to prevent and manage disease epidemics.

This work was supported by an Australian Research Council Linkage grant, the Department of Primary Industries, Parks, Water and Environment and Zoos SA. J.J.A. and K.B. are supported by ARC Future Fellowships. K.M. is supported by an Australian Postgraduate Award. We thank the Western Australian Museum, Museum Victoria Queen Victoria Museum and Art Gallery, Tasmanian Museum and Art Gallery, American Museum of Natural History, Leeds Museum, and Oxford University Museum for access to historical museum specimens.

References


