5.6. Trypanosomes

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Abstract

Trypanosomes of the order Kinetoplastida are introduced within a framework of their biological diversity and ecological impact. A morphologically distinct trypanosome species was detected by light microscopy at relatively high prevalence level of ~ 40% within the declining woylie population of the Upper Warren region. Initial investigation by light microscopy revealed no trypanosomes within the stable and fenced woylie population within the Karakamia Wildlife Sanctuary (operated by Australian Wildlife Conservancy). Further investigation employing molecular techniques revealed the trypanosome within the Upper Warren area to be novel based on analysis of the 18SrRNA gene, and also to be present within the Karakamia population regardless of initial microscopy results. The potential for trypanosomes to negatively impact on host fitness, either as a singular infection whilst the host experiences additional environmental stress, or as part of a concomitant infection together with *Toxoplasma gondii*, is discussed.

5.6.1. Introduction

Protozoan trypanosomes of the order Kinetoplastida are blood parasites primarily of insects but are known to infect a wide range of mammals, birds, reptiles, amphibians and fish. Some species, such as *T. congolense* and *T. vivax* of cattle are economically significant in many developing nations, with infection resulting in death or reduced output and reproductive capacity through emaciation and anaemia. Infection by the trypanosomes *T. gambiense* and *T. rhodesiense* lead to human trypanosomiasis, otherwise known as sleeping sickness in Africa, whereas infection with *T. cruzi* in South America is known as Chagas disease. All three forms of trypanosomiasis result in chronic infections that can be fatal if not properly treated. In all the above cases, the principal route of infection is through an obligate intermediate host or insect vector such as the tsetse fly in Africa and triatomid bugs in South America.

Many wildlife species are commonly infected with endemic or host-specific trypanosome species with little or no apparent pathological effects. However, infection with novel species often has fatal or significantly debilitating effects. For example, *T. lewisi*, a trypanosome associated with the black rat (*Rattus rattus*) has been implicated in the decline of native rodent species along an invasion front in Madagascar (Laakkonen, 2002; Laakkonen *et al.,* 2003). Pickering and Norris (1996) have also implicated the arrival of *R. rattus* onto Christmas Island and the subsequent spread of *T. lewisi* in the decline and extinction of the endemic rat *R. macleari*. Experimental infections involving mice and voles have resulted in much faster and significantly higher parasitemia rates when non-host specific trypanosomes were inoculated compared to what was observed during infection with a host specific trypanosome species (Maragi *et al.,* 1989). There is also strong evidence to suggest that virulence in trypanosome infection is in part host-condition dependent, with individuals in poor condition as a result of concomitant infections, stress due to resource shortage or even low genetic diversity, more likely to suffer negative effects than healthy individuals (Brown *et al.,* 2000). Furthermore, concomitant infections have also been shown to be affected, with rats experimentally infected with *T. lewisi* showing higher levels of *Toxoplasma gondii* tachyzoites at periods of four and seven days post infection than uninfected control animals (Guerrero *et al.,* 1997; Cox, 2001).

In the present context, trypanosomes are under consideration as a contributory factor in the recent declines of the woylie (*Bettongia penicillata*). There are several factors that suggest a possible role for trypanosomes including reduced survival and increased mortality rather than emigration or poor recruitment as the likely primary mechanisms for population decline. It is also possible that some underlying factors may be increasing the vulnerability of individuals to predation (Chapter 2 UW Fauna Monitoring and Section 4.3 Survival and mortality). In addition, trypanosome infection may be compounding the effects of concomitant infections as mentioned

above. It is particularly relevant to note the synergistic effects of trypanosomes when in combination with *Toxoplasma*, a highly pathogenic infection spread primarily by cats and which has been confirmed within the Upper Warren woylie populations (Section 5.7 *Toxoplasma*). The spread of a novel trypanosome species must also be considered in any investigation. Trypanosome vectors of small mammals are generally fleas or ticks or some other blood-sucking insect such as mosquitoes or biting flies. The possibility exists that a non-native trypanosome, possibly originating from a non-native invasive host species such as a cat, fox, rodent or pig etc, has become established within the woylie ectoparasite fauna of the region. It is also possible that a novel ectoparasite vector has jumped host and is now maintaining a novel trypanosome parasite-host cycle within the woylie population.

5.6.2. Methods

Light microscopy has traditionally been used to determine the presence, absence and relative morphology of trypanosomes in appropriately stained blood smears. However, the recent trend has been to adopt more sensitive and specific molecular methods based on DNA amplification by PCR (polymerase chain reaction) to determine presence or absence of infection, and DNA sequencing and genotyping for characterisation and identification to species level. The gene targeted for the present study was a variable region of the trypanosome 18SrRNA gene, with a full description of the techniques used, together with primer details given by Smith *et al.* (2005).

5.6.3. Results

Light microscopy revealed an overall trypanosome prevalence of 43% (n = 45) in a sample of 105 woylies from the Upper Warren, and zero prevalence in a sample of 25 woylies from the Australian Wildlife Conservancy-operated Karakamia population. However, amplification of the small ribosomal RNA subunit (18SrRNA) gene resulted in the detection of two trypanosome-infected animals out of a subset of six individuals re-tested from the Karakamia population that was previously assumed to be trypanosome-free (Figure. 5.6.1).

Figure 5.6.1. Trypanosome DNA amplification using primers specific to the 18SrRNA gene. Lane 1: molecular marker 1KB; lanes 2-7: Woylie samples from the Karakamia population; Lane 8: positive control.

Sequencing of the positive samples from Karakamia and Upper Warren suggests they are more closely related to each other than any other trypanosome species in the database, but also closely related to a trypanosome species found in a wombat and in a potoroo (T. sp P63) (Hamilton *et al.*, 2005). Another novel trypanosome species isolated from a chuditch (*Dasyurus geoffroii*) from

Julimar, and most closely related to *T. bennetti* found originally in a kestrel in North America and prevalent in birds, has been included in the phylogenetic tree in Figure 5.7.2. Sequencing of the amplified trypanosome DNA was carried out using NCBI (National Centre for Biotechnology Information) BLAST (Basic Local Alignment Search Tool) programme. The phylogenetic tree was constructed using the programme Mega 4.0, neighbour-joining, 1000 bootstrap with default parameters.
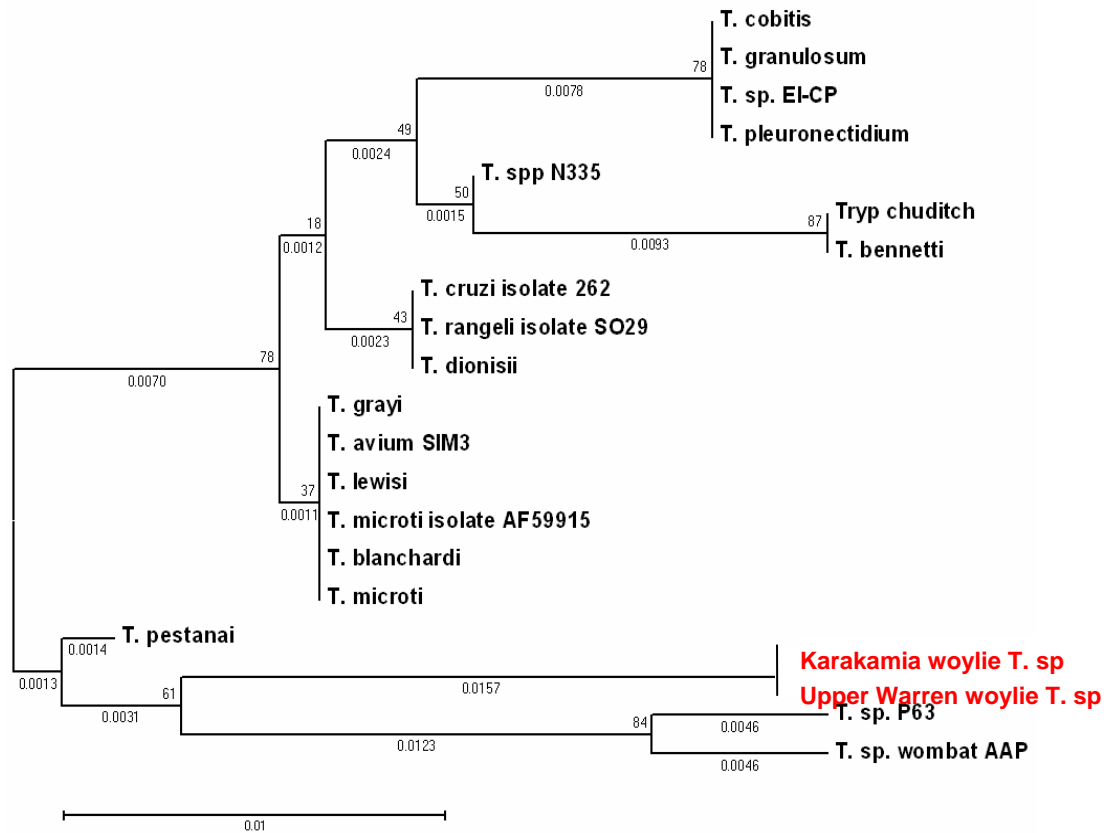

Figure 5.6.2. Phylogenetic tree based on bootstrap analysis of the 18SrRNA gene from 20 trypanosome sequences. Branch numbers represent bootstrap values. The clade that includes the Karakamia woylie trypanosome also includes trypanosomes found in other native species. The trypanosome found in a chuditch sample falls in a clade representing trypanosome species commonly found in birds.

Out of a further subset of five previously untested samples from the Upper Warren population four were found to be positive for trypanosome infection by PCR (Figure 5.6.3). We are currently in the process of DNA cloning and further sequence analysis to determine the genotypes of the trypanosome species present within each of the populations.
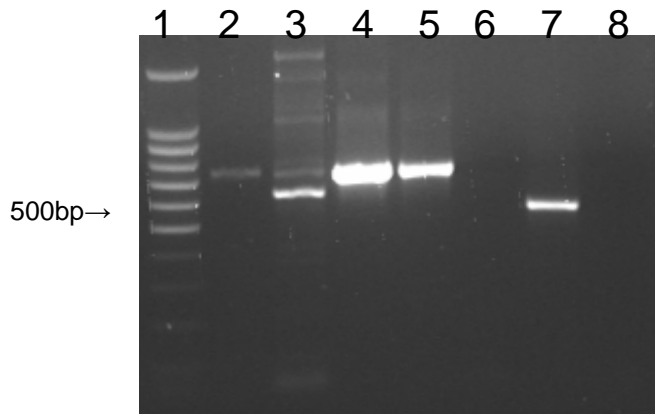
Figure 5.6.3. Amplification of the trypanosome 18S rRNA gene from woylie samples collected in the Upper Warren area. Lane 1: Molecular marker 100bp; Lane 2-6: woylie samples; Lane 7: positive control; Lane 8: negative control.

5.6.4. Discussion

Trypanosome prevalence, at 43% positive by light microscopy, appears relatively high within the Upper Warren woylie population, with further investigations using PCR based methods likely to reveal even higher levels (Desquesnes and Davila, 2002; Solano *et al.,* 1999). With little baseline data to refer to it is not possible to say whether this prevalence level is within a normal range or not, but it is in stark contrast to the zero prevalence level reported from within the non-declining Karakamia population.

There are several additional factors that must be taken into account when considering the potential role and effect of trypanosomes in the woylie decline. One of the most important is the prevalence and diversity of concomitant infections within the population. Within the Karakamia population, PCR-based methods have indicated that, contrary to light microscopy, there are some individuals positive for trypanosome infection. However, as reported in section 5.7, all samples screened so far (n = 81) for *Toxoplasma* using the modified agglutination test have been sero-negative, whereas the minimum prevalence within the Upper Warren population during March 2006 was 5.8% (Section 5.7 *Toxoplasma)*. As mentioned above, the effects of *Toxoplasma* have been shown to be more severe (increased tachyzoites count) when the host was also co-infected with trypanosomes. These synergistic effects are not clearly understood and, furthermore, it is not known whether such mixed infections adversely affect the impact or pathogenicity of trypanosomes.

There is also evidence to suggest that, even when considered as a singular infection, trypanosomes can have a direct negative impact on host fitness (Brown *et al.,* 2000). For example, Ratti *et al.* (1993) showed that male pied flycatchers (*Ficedula hypoleuca*) that were infected with a *Trypanosoma* species had poorer feather development and arrived at their destination several days later than uninfected males following their annual migration. If trypanosome infected woylies were to suffer a slight reduction in fitness, perhaps in a way that resulted in their becoming more prone to predation or the effects of other diseases in the environment, then that would amount to a significant effect at the population level considering the high overall prevalence of trypanosome infection reported above.

Aside from differences in prevalence levels and concomitant infections, there remains the possibility that a novel trypanosome species has been introduced into the region. This remains highly speculative at present until genotyping and sequencing results become available. However,

initial sequencing results have indicated little difference between trypanosome species from either population which further suggests an additive effect of concomitant infections or environmental stress.

5.6.5. References

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