

## Genotypes and nematode infestations in an endangered lizard, *Tiliqua adelaidensis*

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### Abstract

We used data from 16 polymorphic microsatellite loci from the endangered pygmy bluetongue lizard, *Tiliqua adelaidensis*, to examine genetic signals of intestinal parasite infestation. 30% of 70 individuals had detectable infestations of the nematode parasite *Pharyngodon wandillahensis*. We found no evidence that higher levels of heterozygosity promoted parasite resistance, and there were no significant associations between 50 common alleles and infestation status. Although neutral markers are a potentially useful conservation tool for studying the risk of parasitism in species of conservation concern, we did not detect any association in the studied host-parasite system.

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### Key words

Conservation, heterozygosity, microsatellites, nematode, reptile, *Tiliqua adelaidensis*.

The conservation management of endangered species should aim to minimise adverse impacts of both endemic and exotic parasites. The risk of extinction to a population may be decreased by a reduction in both parasite prevalence and intensity (Thorne and Williams, 1988). These parameters of parasite distribution can be influenced by genetic variation among individuals in host populations (Wasom et al., 1986). Thus, any genetic signal of increased resistance will be a useful conservation tool. Microsatellite DNA is increasingly being used as a tool to interpret ecology, behaviour and genetic structure of populations, including those of endangered species. It may also provide genetic signals of parasite resistance (e.g. Coltman et al., 1999).

Two components of the microsatellite genotype could potentially provide that signal. The first is the level of heterozygosity in the genotype. Small isolated populations of an endangered species may become inbred, leading to reduced heterozygosity. The major histocompatibility complex (MHC) is a genomic region

involved in immune responses and disease resistance in vertebrates (Doherty and Zinkernagel, 1975). MHC genes are characterised by extremely high levels of polymorphism which indicate an adaptive advantage for allelic diversity (Hedrick, 1994; De Bellocq et al., 2008). Studies on MHC variation in a range of animal species have found high allelic diversity to be associated with protection from parasites (Paterson et al., 1998; Kurtz et al., 2003). Because genome-wide heterozygosity is important for parasite resistance (Luong et al., 2007), increased heterozygosity at microsatellite loci may provide a useful measure of functional genetic diversity in other parts of the genome, including the MHC. For example, Coltman et al. (1999) reported significantly lower levels of intestinal parasitism in wild soay sheep with higher microsatellite heterozygosity.

Secondly, while specific MHC genes directly influence parasite resistance or susceptibility (e.g. Westerdahl et al., 2005), microsatellite loci may be physically linked to genes that affect host resistance to pathogens (either within the MHC, or from other parts of the genome). Neutral microsatellites alleles can therefore be used in studies of disease resistance (Wiener et al., 2003). For example, Godfrey et al. (2006) reported a significantly lower prevalence of blood parasites for individuals with a particular microsatellite allele in a wild population of the Australian gidgee skink, *Egernia stokesii*.

The pygmy bluetongue lizard, *Tiliqua adelaidensis* is an endangered skink (IUCN, 2007) now restricted to a few isolated fragments of native grassland around Burra, in South Australia (33°41'S, 138°56'E) (Souter et al., 2007). We examined the infestation pattern of the recently described intestinal parasite *Pharyngodon wandillahensis* (Nematoda) (Fenner et al., 2008) among individual *T. adelaidensis*. Our aim was to determine whether genotypes of neutral genetic markers were associated with infestation prevalence or intensity. Nematode parasites can have negative effects on the fitness of individuals in wild populations, ranging from reduced vigour to increased mortality (Coltman et al., 1999). Although oxyurid nematodes like *Pharyngodon* are not usually agents of morbidity, Fenner and Bull (2008) showed that they reduced activity levels in a related Australian skink. If nematode parasitism is associated with host genotype in *T. adelaidensis*, microsatellite markers could be used to examine the impact of parasites on the fitness of this endangered lizard.

Over the period from September 2005 to March 2006, we sampled 70 adult lizards from three populations within a 10 km radius of Burra. This was a subset of the sample of lizards collected for analysis in Gardner et al. (2008) and included those lizards that produced a scat during sampling. The lizards inhabit burrows constructed by lycosid and mygalomorph spiders within native tussock grasslands dominated by *Lomandra* sp. (Hutchinson et al., 1994). The study sites, which all occur on private land, are subject to sheep grazing and include a large component of exotic plant species. Each lizard was toe clipped for individual identification and blood from the toe was transferred to FTA<sup>®</sup> paper (Whatman) for DNA storage. DNA was later extracted from the FTA<sup>®</sup> paper (Smith and Burgoyne, 2004) and

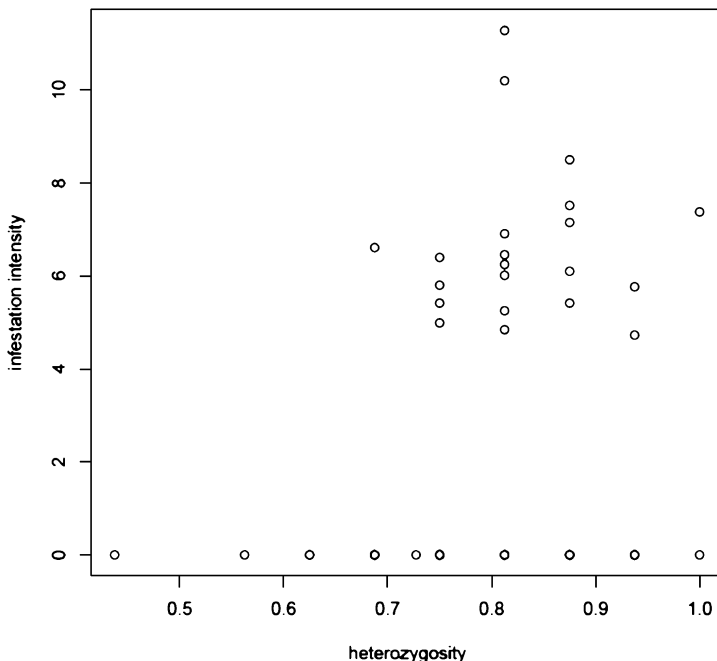
each lizard was genotyped for 16 polymorphic microsatellite loci (Cooper et al., 1997; Gardner et al., 1999; Gardner et al., 2008). Heterozygosity was calculated as the proportion of the 16 loci that were heterozygous in each individual.

During capture, fresh scats were collected and stored in Sodium Acetate Formalin. We determined the infestation status of each individual by assessing the presence or absence of nematode worms or eggs in scats. Parasite intensity in infested individuals was assessed by estimating the number of eggs per gram of scat (Gordon and Whitlock, 1939). This may not reflect actual adult parasite load, but is the best non-invasive technique available, and thus appropriate for surveying intestinal parasites of endangered species. Several factors may lead to false negative readings of prevalence or biased estimates of infestation intensity, including a pre-patent period for worms, or irregular or seasonal shedding patterns for eggs. However, both laboratory and field measures of the congeneric *Pharyngodon tiliquae* showed a consistent shedding of eggs in scats of the lizard *Egernia stokesii* over several months during the lizard activity season (Fenner and Bull, 2008).

We detected nematode infestations in 21 (30%) of the 70 sampled lizards. Prevalence did not vary among the three sampled sites (24.3% of 37 lizards, 39.1% of 23 lizards and 30.0% of 10 lizards:  $\chi^2 = 1.48$ ; d.f. = 2;  $P = 0.48$ ). Mean heterozygosity in the 21 infested lizards was 0.83 (SE = 0.02) and in the 49 uninfested lizards was 0.79 (SE = 0.01). Among the 21 infested lizards, heterozygosity ranged from 0.68 to 1.00, and estimated infestation intensity ranged from 100 to 100,000 nematode eggs  $g^{-1}$  of scat. However there was no significant correlation between these two parameters (Spearman's rank correlation:  $r = 0.114$ ;  $P = 0.62$ ). The correlation was stronger when uninfested individuals were included in the analysis, but the result was not significant (Spearman's rank correlation:  $r = 0.201$ ;  $P = 0.10$ ; fig. 1). A generalised linear model with a logit link function showed that parasite infestation status did not differ between sample sites ( $P = 0.83$ ) and was not influenced by heterozygosity ( $P = 0.22$ ), or the interaction between heterozygosity and sample site ( $P = 0.90$ ).

There was no pattern to suggest that higher levels of heterozygosity promoted higher resistance to infestation from nematodes in our sample. Intermediate levels of genetic variation may be optimal for parasite resistance (Kurtz et al., 2003), but we found no evidence of this when we modelled infestation intensity as a function of the squared term of heterozygosity (multiple linear regression:  $F_{3,66} = 1.23$ ,  $P = 0.61$ ). We are currently unable to separate two alternative explanations for the lack of association. First, a panel of less than 20 microsatellite markers may have insufficient power to reflect genome-wide heterozygosity (Ortego et al., 2007). Second, a lack of association between genetic diversity and parasite prevalence could indicate the limited fitness costs of the parasite on the host (Ortego et al., 2007). However, further ecological studies of *P. wandillahensis* are necessary to examine its impact on *T. adelaidensis*.

In a second analysis, we examined 50 common alleles that were each found in >10% of the sample (2–5 alleles per locus). We used chi-square tests to investi-



**Figure 1.** Relationship between infestation intensity ( $\log(\text{eggs g}^{-1} \text{ scat} + 1)$ ) and heterozygosity in 70 pygmy bluetongue lizards.

gate whether the frequency of each allele differed between infested and uninfested lizards, and found no significant associations. There was no need to adjust  $P$  values for multiple tests to reduce the chance of type I errors because there were no chi-squared tests with a  $P < 0.05$ . This result does not tell us that resistance to infestation from nematodes is not genetically determined in *T. adalaidensis*. It simply shows that in the sample of 16 loci that we explored, none were sufficiently closely linked to a resistance gene to show any pattern of allelic association.

In summary, our survey of three populations of the endangered pygmy bluetongue lizard failed to detect any genetic signal of resistance to a nematode parasite, either in levels of heterozygosity or in allele frequencies, using microsatellite DNA analysis. Thus, although neutral markers are a potentially useful conservation tool for studying the risk of parasitism in species of great conservation concern, we could not detect any association between microsatellite genotypes and parasitism in the studied system.

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