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Disease induced changes in gene flow patterns among Tasmanian devil populations

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ABSTRACT

Infectious diseases of wildlife reduce population size and may erode genetic diversity, constituting an extinction threat. The Tasmanian devil (*Sarcophilus harrisii*) is threatened with extinction by an infectious cancer, the devil facial tumour disease (DFTD). In less than two decades, DFTD has caused a more than 85% overall population decline. We used ten polymorphic microsatellite loci to quantify the effects of this decline on genetic diversity, population differentiation, effective population size, and gene flow. Samples from 1999 and 2006 at five locations were analysed, three of which had been affected by DFTD during this time interval. Significant increases in inbreeding coefficient (f) and non-significant reductions in effective population size were observed for both diseased and non-diseased populations, and therefore there was no consistent effect of DFTD. There was significant but stable structuring of genetic variation among locations through time, although a dynamic "source-sink" relationship was evident for gene flow associated with disease-mediated changes in population densities. These changes in gene flow may have contributed to the maintenance of genetic diversity in disease-affected areas. Simulations suggest that the estimated population declines, although severe, have been insufficient to yield significant changes in genetic variation; this may have been exacerbated by disequilibrium between population sizes and genetic diversity at the time of DFTD emergence, owing to elevated devil abundances following the extinction of the previous apex predator—the thylacine—approximately 80 years ago.

1. Introduction

Emerging infectious diseases of wildlife are recognized as a significant threat to global biodiversity (Smith et al., 2009). They have been shown to reduce population size (N) (Robinson et al., 2010), alter spatial genetic population structure (Lee et al., 2010), population age structure (Lachish et al., 2009), life-history parameters (Jones et al., 2008), and dispersal patterns (Hurtado, 2008), all factors that can compromise a species' long-term survival (Frankham, 2005). Low genetic diversity is common in threatened species (Spielman et al., 2004) and is often caused by population reductions driven by factors such as habitat degradation or fragmentation, over-hunting, or climate change (Chiucchi and Gibbs, 2010; Hansen et al., 2009; MacDonald et al., 2008). The genetic consequences of these population declines may compromise species' resistance to disease (Altizer et al., 2003; Lee et al., 2010), which in turn, can lead to further reductions in population size and genetic diversity.

The world's largest marsupial carnivore, the Tasmanian devil (*Sarcophilus harrisii*), is currently facing extinction threat caused by the transmissible cancer, devil facial tumour disease (DFTD). DFTD is derived from Schwann cells in the peripheral nervous system (Murchison et al., 2010) and is restricted to a single host, the Tasmanian devil. It manifests as facial tumours and is consistently fatal, usually within 6 months of infection (Jones et al., 2008; Lachish et al., 2007). Cancers are not usually infectious—they typically arise and die within a single host (Hanahan and Weinberg, 2000)—and the only other known case in which live tumour cells are infectious is canine transmissible venereal tumour (Murchison, 2009). Intimate, injurious contact is the route for transmission of live tumour cells (McCallum and Jones, 2012; Murchison, 2009). Genetic diversity was low in Tasmanian devils prior to the emergence of DFTD for both the functional MHC genes (Cheng et al., 2012; Morris et al., 2013; Siddle et al., 2007), and for mitochondrial- and nuclear loci (Jones et al., 2004; Miller et al., 2011; Siddle et al., 2010). Reduced MHC diversity preceded the isolation of Tasmania with sea level rise ~13,000 years ago (Morris et al., 2013; Siddle et al., 2013). Although low genetic diversity may have played a role in the evolution of transmissibility, both the devil and the dog cancers have evolved sophisticated mechanisms to evade the immune

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system of the host, involving down-regulation of MHC expression in the tumour (Fassati and Mitchison, 2010; Murgia et al., 2006; Siddle et al., 2013).

Currently, the spread of DFTD is reducing devil population size (DPIWE, 2012; Hawkins et al., 2006; McCallum et al., 2007), but with unknown effects on its already low genetic diversity. Since DFTD was first detected in 1996 at Mt. William National Park in north-eastern Tasmania, it has spread to the majority of the species' range, causing more than 85% overall population decline, with local declines in excess of 95%, contributing to "Endangered" listing status (Hawkins et al., 2006; IUCN, 2010; McCallum et al., 2007). DFTD transmission is strongly frequency dependent (McCallum et al., 2009, 2007), creating a risk of disease-driven extinction because transmission is sustained even at very low population densities through the requirement of contact for reproduction (McCallum, 2008; Smith et al., 2009). However, geographic spread of DFTD is likely to be slower than gene flow, as adults (which are the predominant infectious host) are restricted to a home range, while juveniles (which are seldom infected with DFTD) move greater distances away from their natal site to establish their own territory (Jones et al., 2008). Natal dispersal in devils tends to be male biased, perhaps driven by inbreeding avoidance, a pattern common in most carnivores (Gachot-Neveu et al., 2009; Goltsman et al., 2005).

Previous studies of genetic diversity in the Tasmanian devil have either concentrated on spatial variation (Jones et al., 2004; Miller et al., 2011; Siddle et al., 2010), or have tested for temporal changes at diseased sites without comparison to control sites that remained uninfected during the same interval (Lachish et al., 2011). In this paper, we investigate changes in genetic diversity through time at sites that became DFTD infected, relative to changes at sites that did not. We ask the following specific questions: (i) Does genetic diversity change through time and could any changes be attributable to DFTD-induced population declines? (ii) Does genetic population structure, sex-biased dispersal and population connectivity change over time and with respect to DFTD mediated changes in population density? (iii) Has DFTD had a detectable effect on effective population size (N_e)? We interpret our results with respect to the future conservation—and particularly the genetic management—of this species.

2. Materials and methods

2.1. Study area and samples

Ear biopsies from Tasmanian devils were collected in 1999 ($n = 213$) and 2006–2007 ($n = 212$) at five locations (Marrawah, Narawntapu National Park, the Freycinet Peninsula, Little Swanport, and Pawleena) in Tasmania (Fig. 1; total sample sizes in Table 1). Devils were trapped using 30 cm diameter PVC pipe traps baited with meat. Forty traps were set across 25 km^2 study areas at landscape locations that carnivores were likely to encounter during nightly movements, with the exception of Freycinet (60 traps in 100 km^2) and Marrawah (40 traps along a 40 km length of road). Traps were checked daily starting in the early morning. All devils were micro-chipped for individual identification and an ear biopsy taken from each individual. To minimize relatedness, only samples from adults (2 + years old) and independent sub–adults (1 year old) that were beyond natal dispersal age were included in analyses. To analyse the effects of DFTD, we sampled pre-disease and post-disease at Little Swanport, Freycinet, and Pawleena, where DFTD arrived in 1999, 2001, and 2002, respectively (Hawkins et al., 2006; McCallum et al., 2007). For non-diseased locations we surveyed Marrawah and Narawntapu over the same time interval. The first reported DFTD case in Narawntapu was in 2007, which is outside our sampling period, and Marrawah is presently disease-free. We also estimated gene flow using samples taken during 2006 from Dunalley ($n = 29$, DFTD arrival = 2004) and the Forestier Peninsula ($n = 75$, DFTD arrival = 2004). This provided a replicate to examine directionality of gene flow in adjacent populations with different disease effects (Dunalley exhibited substantial declines at the time of sampling, while Forestier exhibited minor declines; Fig. 1; McCallum et al., 2007). We will refer to the period "2006–2007" as "2006" throughout.

2.2. Genotyping

Each individual was genotyped for ten polymorphic microsatellite loci developed for Tasmanian devils following Jones et al. (2003): *Sh2v* (1), *Sh2p* (1), *Sh3o* (1), *Sh6l* (1), *Sh6e* (1), *Sh2i* (1), *Sh2g* (2), *Sh2l* (3), *Sh5c* (6), and *Sh3a* (not analysed), with assignment to each of $n = 7$ chromosomes given in parentheses. All samples from 1999 were genotypes taken from Jones et al. (2004). The 2006 samples from Marrawah and Narawntapu were genotyped during this study, as were subsets of the Freycinet ($n = 28$), Dunalley ($n = 8$) and Forestier ($n = 73$) samples. Remaining 2006 Little Swanport, Pawleena and subset-parts of the Freycinet ($n = 20$), Dunalley ($n = 21$) and Forestier ($n = 2$) samples where genotyped by Lachish et al. (2011). We did not re-genotype any samples.

2.3. Tests for genotyping errors and selection

Data were checked for scoring errors associated with allele stuttering, allele drop-out, and null alleles using MICRO–CHECKER ver. 2.2.3 (Van Oosterhout et al., 2004). Tests for selection was performed using the Bayesian approach in BAYESCAN ver. 2.01 (Foll and Gaggiotti, 2008), which is an extension of Beaumont and Balding's (2004) method. This allows differences in allele frequencies between populations and their ancestral common gene pool, and differences in F_{ST} to be interpreted in light of a demographic model where differences in effective population size and gene flow may contribute in addition to selection (Foll and Gaggiotti, 2008). Posterior odds (PO) are used to evaluate how much more likely the model with selection is compared to the neutral model, and allows the user to control the false-positives rate. BAYESCAN can handle small sample sizes, as it incorporates uncertainty in allele frequencies, without risk of estimate bias. A Reversible Jump Markov chain Monte Carlo (RJ-MCMC) algorithm was used to obtain posterior distributions of the degree of differentiation (F_{ST}) decomposed into a locus-specific component (α), shared by all populations, and a population-specific component (β), shared by all loci. Selection is assumed when α is necessary for explaining the observed pattern of diversity. We tested the two datasets (1999 and 2006) separately using sample size = 5×10^3, thinning interval = 10^2, pilot runs = 10^2, pilot run length = 10^4, and additional burn-in = 5×10^5. These are higher than the default settings, which normally ensure good convergence in most cases (Foll and Gaggiotti, 2008). Convergence of the RJ-MCMC was tested by comparing the sample means of an early segment (first 10% of the RJ-MCMC) and a later segment (last 50% of the RJ-MCMC) for significant deviation (Geweke, 1992), in R ver. 2.12.2 (2011) using the BOA package (Smith, 2007). We used PO of 10 and 100, corresponding to accepting a false-positive rate of 5% and <1%, respectively, to make decisions on whether a locus was under selection (positive or balancing) (Foll and Gaggiotti, 2008). The two PO values are interpreted as "substantial" and "very strong" evidence for selection, respectively (see BAYESCAN program notes for details). The PO values for each locus were calculated in R and outliers identified.