Inbreeding depression in one of the last DFTD-free wild populations of Tasmanian devils

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Abstract

Background. Vulnerable species experiencing inbreeding depression are prone to localised extinctions because of their reduced fitness. For Tasmanian devils, the rapid spread of devil facial tumour disease (DFTD) has led to population declines and fragmentation across the species' range. Here we show that one of the few remaining DFTD-free populations of Tasmanian devils is experiencing inbreeding depression. Moreover, this population has experienced a significant reduction in reproductive success over recent years.

Methods. We used 32 microsatellite loci to examine changes in genetic diversity and inbreeding in the wild population at Woolnorth, alongside field data on breeding success from females to test for inbreeding depression.

Results. We found that maternal internal relatedness has a negative impact on litter sizes. The results of this study imply that this population has entered an extinction vortex and that to protect the population, genetic rescue may be required. This study provides conservation managers with useful information for managing wild devils and provides support for the "Wild Devil Recovery Program" which is currently augmenting small, isolated populations.

Introduction

For threatened species, a reduction in reproductive success can severely impact population persistence. The Tasmanian devil, *Sarcophilus harrisii,* is one such species that has a decline of up to 80% in areas infected by an infectious clonal cancer, devil facial tumour disease (DFTD) (Loh *et al.* 2006; Pye *et al.* 2016; Lazenby *et al.* 2018). As the apex carnivore in Tasmania, devil population declines are causing trophic cascades in the Tasmanian ecosystem (Hollings *et al.* 2014) and recent modelling has indicated that these populations will begin to succumb to small population genetic pressures (Grueber *et al.* 2018). Declining populations are at risk of reduced

37 gene flow and loss of genetic diversity (relative to larger, more connected populations) as an
38 outcome of genetic drift and inbreeding (Charlesworth & Willis 2009).
39   Since the discovery of DFTD in the mid-1990s, the national and international
40 conservation community has rallied and research into Tasmanian devil biology has grown
41 rapidly, including studies of DFTD epidemiology (e.g. Hamede *et al.* 2008; McCallum *et al.*
42 2009; Hamede *et al.* 2012), devil behaviour (e.g. Sinn *et al.* 2014), ecological impacts (e.g.
43 Hollings *et al.* 2014), population genetics (e.g. Lachish *et al.* 2011; Grueber *et al.* 2015; Epstein
44 *et al.* 2016; Hendricks *et al.* 2017), *ex situ* conservation (e.g. Hogg *et al.* 2016) and
45 translocations (e.g. Rogers *et al.* 2016; Thalmann *et al.* 2016; Wise *et al.* 2016; Grueber *et al.*
46 2017). As DFTD spread from the north-east across Tasmania, devil populations have been
47 monitored by the Save the Tasmanian Devil Program (STDP) since 2004 (Lazenby *et al.* 2018).
48 One of the last-known DFTD-free populations is located at Woolnorth (40.77° S, 144.77° E),
49 north-west Tasmania (Farquharson *et al.* 2018; Lazenby *et al.* 2018). Since 2014, this
50 population has suffered an extreme decline in reproductive output, the cause of which remains
51 unclear (Farquharson *et al.* 2018).
52   Here we aimed to test whether the observed decline in wild devil reproductive fitness
53 (specifically litter sizes) is a result of inbreeding depression. Inbreeding depression occurs when
54 an accumulation of deleterious recessive alleles lowers individual heterozygosity, negatively
55 impacting individual fitness relative to less-inbred individuals or populations (Keller & Waller
56 2002; Frankham *et al.* 2017). Previous genetic research on a captive Tasmanian devil
57 population revealed inter-individual variation in inbreeding, but no signs of inbreeding
58 depression (Gooley *et al.* 2017). Although inbreeding depression is easier to study in controlled
59 environments (such as captivity), it may be more consequential in the wild, as environmental
60 conditions are more severe (Joron & Brakefield 2003; Armbruster & Reed 2005; de Boer *et al.*
61 2015). Thus, studies of inbreeding depression in captive environments may underestimate the
62 impact on inbreeding on fitness in the wild (Kristensen *et al.* 2008; Gooley *et al.* 2017). In
63 addition, wild populations that experience inbreeding depression are more vulnerable to
64 extinction (Keller & Waller 2002), and so isolated populations may need genetic rescue to
65 combat the effects of inbreeding (Frankham 2015; Frankham *et al.* 2017).
66   Here we use multilocus heterozygosity to investigate inbreeding and inbreeding
67 depression at the DFTD-free population of devils at Woolnorth. We aimed to test: 1) whether
68 inbreeding is occurring in the devil population at Woolnorth, and 2) whether inbreeding is
69 associated with the observed reduction in reproduction (specifically litter sizes). The results of
70 this study will inform the ongoing management of fragmented devil populations in the face of
71 DFTD.

72

73 Materials & Methods

74 *Sample collection and genotyping*

75 Samples were collected by the STDP following their Standard Operating Procedure (see
76 Appendix 5 in Hogg *et al.* 2019) and shared with the University of Sydney for genetic analysis.
77 DNA samples and corresponding reproductive and demographic data were available for years
78 2006, 2007, 2009, 2014, 2015 and 2016. Reproductive output for females was taken as the

79 estimated count of offspring produced (i.e. "litter size"), following Farquharson et al. (2018).
80 Female devils are limited to a maximum of 4 offspring per breeding event (Guiler 1970). For our
81 data, litter size was estimated either by the presence and count of pouch young, or, for
82 monitoring trips that occurred later in the year, by the presence and count of active teats
83 (indicating pouch young had been denned) (following Keeley *et al.* 2012; Farquharson *et al.*
84 2018). In total, 168 wild Tasmanian devils (90 females and 78 males) were included in this
85 study. Male reproductive output could not be examined in this study.
86   DNA from ear biopsy samples from the 2006, 2007 and 2009 monitoring trips had been
87 previously extracted (Hendricks *et al.* 2017), whilst samples from 2014, 2015 and 2016 were
88 extracted using a phenol-chloroform technique (Sanbrook *et al.* 1989) and stored at -20°C.
89 Samples were genotyped with 32 neutral microsatellite markers following Gooley *et al.* (2017)
90 and Jones *et al.* (2003). A randomly chosen set of 7% were re-genotyped to estimate
91 genotyping error. We tested for null alleles at each locus using Micro-Checker (van Oosterhout
92 *et al.* 2004). GenAlEx (Peakall & Smouse 2006, 2012) was used to calculate observed (H_O) and
93 expected heterozygosity (H_E) for each locus, each year, and conduct Hardy-Weinberg exact
94 tests.

95 *Inbreeding and inbreeding depression*

96 Internal relatedness (IR), a multilocus heterozygosity statistic that is expected to be positively
97 correlated with individual inbreeding coefficient (Amos *et al.* 2001), was calculated using the
98 package *Rhh* (Alho *et al.* 2010) for R (R Core Team 2018). IR incorporates allele frequencies,
99 because there is a higher chance that rare-allele homozygosity is the result of inbred mating,
100 relative to common-allele homozygosity (Amos *et al.* 2001). All available samples, male and
101 female, were used to estimate allele frequencies. We examined whether inbreeding was
102 accumulating by testing for a change in IR over time using a linear model fitted in R with year as
103 the fixed predictor and IR as the response (N = 168).
104   To interpret associations between heterozygosity and litter sizes as inbreeding
105 depression, molecular data must reflect variation in inbreeding levels among individuals, i.e.
106 identity disequilibrium (Szulkin *et al.* 2010). This variation was quantified with the g_2 statistic
107 (David *et al.* 2007; Szulkin *et al.* 2010), using the package *inbreedR* (Stoffel *et al.* 2016) for R,
108 with its precision evaluated using 1,000 Monte Carlo iterations.
109   We tested for inbreeding depression by determining whether IR was a predictor of
110 female litter size using linear regression; we predict a negative slope (i.e. increased IR is
111 associated with decreased litter sizes). Litter size was modelled as a binomial response, where
112 the number of events (successes) equalled the inferred litter size (based on pouch status; N =
113 90 unique females), and the number of trials equalled the maximum possible litter size of four.
114 Along with IR (our predictor of interest), age (based on tooth wear observations, Pemberton
115 1990) and year were also included as continuous fixed predictors (with year = 0 for 2006).

116 **Results**

117 We found no evidence of null alleles at any of our loci, and missing data was low: >90% of
118 individuals were successfully genotyped for >90% of loci. Genotyping error rate was 0.6%.
119 Microsatellite diversity of Woolnorth devils was low (Table 1), and similar to observations of

120 other wild sites and captive populations (e.g. Gooley *et al.* 2017; Storfer *et al.* 2017; Grueber
121 *et al.* 2018). Levels of IR remained constant across the study period (linear regression: β_{Year}
122 = 0.003 ± 0.005 SE, p = 0.546; β_0 = -5.621 ± 9.295 SE, p =- 0.546, N = 168 devils, Figure 1).
123   We detected statistically significant identity disequilibrium in our dataset (g_2 = 0.017,
124 SE = 0.007, p-value = 0.003), indicating that variation at our molecular markers reflects
125 variation in the level of inbreeding among individuals. We found evidence that inbreeding
126 depression is occurring in the devil population at Woolnorth: IR had a statistically significant
127 negative effect on female litter sizes (increased homozygosity [IR] was associated with
128 decreased fitness) (Figure 2; Table 2).

129 Discussion

130 Here, we show that one of the last-known DFTD-free wild populations of Tasmanian devils is
131 experiencing inbreeding depression. Although our data did not detect an increase in inbreeding
132 over the timescale of our study, we did show that maternal IR has a negative impact on
133 reproductive output (litter size) in wild devils. A significant decline in reproduction over time has
134 been observed at Woolnorth (Farquharson *et al.* 2018); inbreeding depression may be either
135 partially responsible for this trend, or a worrying consequence of it. Taken together, these
136 observations suggest that the Woolnorth population may be close to a tipping point, whereby
137 inbreeding reduces reproductive rates (perhaps in concert with other factors), which in turn
138 further reduces population size and exacerbates the occurrence of inbreeding and inbreeding
139 depression. This raises the management option of genetic rescue whereby supplementation
140 could increase the fitness of this population, which is now effectively isolated as a result of devil
141 facial tumour disease causing 80% declines in adjacent devil populations (Whiteley *et al.* 2015;
142 Lazenby *et al.* 2018).
143   Small populations that exist in fragmented landscapes are expected to increase in mean
144 inbreeding levels over time (Wright *et al.* 2007; Frankham *et al.* 2017) and monitoring this
145 process is an important element of genetic management in conservation (Fredrickson *et al.*
146 2007; La Haye *et al.* 2012). Given the short time-period in which litter size appears to be
147 decreasing at Woolnorth (Farquharson *et al.* 2018), our failure to detect a corresponding change
148 in IR over time may indicate that a measurable increase in population mean inbreeding is yet to
149 occur. This interpretation is not unprecedented: for example, the southernmost Swedish
150 population of arctic fox did not show an increase in inbreeding coefficients until four years after
151 population fragmentation that occurred in the late 1990s (Noren *et al.* 2016). In any case, the
152 declining reproductive output seen here, and previously (Farquharson *et al.* 2018), could lead to
153 a decrease in effective population size. If true, the result will be an eventual increase in
154 inbreeding, and a strengthening of its negative effects. To test this hypothesis, it will be
155 important to continue monitoring of the trajectory of demographic and genetic processes
156 occurring in this population, given its importance as the last DFTD-free wild population of
157 Tasmanian devils.
158   Devil populations, with and without DFTD, are fragmented across the landscape, so
159 inbreeding depression may be occurring at other sites, particularly those affected by DFTD. It
160 would be informative to quantify inbreeding depression into the future to facilitate effective
161 management of wild populations. Evidence of inter-individual variation in inbreeding at

162 Woolnorth (g_2 analysis) indicates that we have the molecular tools available to test for
163 inbreeding depression; it will be informative to determine whether this is also true for other sites.
164 The results can be used to predict the outcomes of the STDP management strategy of
165 augmenting small wild populations to promote gene flow (Grueber *et al.* 2018; Fox & Seddon
166 2019).

Conclusions

168 In conclusion, we have presented the first documented evidence of inbreeding depression in a
169 wild population of Tasmanian devils. Whether inbreeding is the driver of the observed
170 reproductive decline at Woolnorth, or the reproductive decline is driving the increase in
171 inbreeding cannot be determined. Nevertheless, our data do show that inbreeding is detrimental
172 in this population, and that it is poised to become more prevalent: this population appears to be
173 at the cusp of the extinction vortex. Augmenting this population with genetic material from other
174 locations across Tasmania may alleviate the effects of inbreeding and minimise inbreeding
175 depression.

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309

Genetic variation of 32 polymorphic microsatellite loci in the Woolnorth Tasmanian devil population.

Genetic diversity measured by number of alleles (Na), observed heterozygosity (H_O),

unbiased estimate of expected heterozygosity (H_E) and Hardy-Weinberg Exact test (p-value).

Total number of devils N = 168.

1 **Table 1**: Genetic variation of 32 polymorphic microsatellite loci in the Woolnorth Tasmanian
2 devil population measured by number of alleles (Na), observed heterozygosity (H_O), unbiased
3 estimate of expected heterozygosity (H_E) and Hardy-Weinberg Exact test (p-value). Total
4 number of devils N = 168.

Locus[1]	N	Na	H_O	H_E	p-value
Sh2b	147	2	0.340	0.378	0.239
Sh2g	167	3	0.701	0.646	0.053
Sh2i	168	3	0.411	0.406	0.443
Sh2p	168	3	0.667	0.617	0.300
Sh2v	168	6	0.548	0.587	0.738
Sh3a	155	3	0.226	0.245	0.078
Sh3o	168	4	0.464	0.522	0.129
Sh5c	160	3	0.069	0.067	0.977
Sh6e	168	2	0.435	0.412	0.452
Sh6L	167	2	0.138	0.139	0.943
Sha001	164	3	0.085	0.083	0.955
Sha008	161	3	0.547	0.534	0.769
Sha009	163	4	0.319	0.297	0.954
Sha010	161	7	0.826	0.778	0.757
Sha011	167	2	0.329	0.386	0.061
Sha012	156	3	0.487	0.538	0.000
Sha013	162	7	0.710	0.675	0.718
Sha014	165	4	0.491	0.525	0.108
Sha015	155	2	0.471	0.471	0.978
Sha023	156	5	0.436	0.423	0.998
Sha024	148	2	0.209	0.199	0.486
Sha025	166	2	0.193	0.231	0.037
Sha026	164	3	0.226	0.233	0.667
Sha028	148	5	0.264	0.241	0.970
Sha033	166	2	0.331	0.301	0.178
Sha034	166	3	0.193	0.200	0.580
Sha036	165	2	0.248	0.295	0.048
Sha037	164	6	0.610	0.688	0.000
Sha039	160	4	0.400	0.407	0.961
Sha040	165	5	0.612	0.599	0.000
Sha042	163	2	0.313	0.297	0.479
Sha032	147	3	0.061	0.060	0.986

5 [1] The ten "Sh" markers were developed by Jones *et al.* 2003; the remaining 22 "Sha" markers
6 were developed by Gooley *et al.* (2017)

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Predictors of the number of joeys (proportion out of a maximum of four; binomial model) produced by female Tasmanian devils (N = 90).

1   **Table 2:** Predictors of the number of joeys (proportion out of a maximum of four; binomial
2   model) produced by female Tasmanian devils (N = 90).

Predictor	Estimate	SE	p-value
Intercept	0.594	0.377	0.116
Age	0.145	0.143	0.312
IR	-2.374	0.680	< 0.001
Year	-0.319	0.038	< 0.001

3   Abbreviations: N = sample size, SE = standard error, IR = internal relatedness

Figure 1

Internal relatedness of Tasmanian devils at Woolnorth (males and females) across years.

Dotted line is at IR = 0. Note: annual monitoring trips were not conducted in 2008, 2010, 2011, 2012 and 2013.
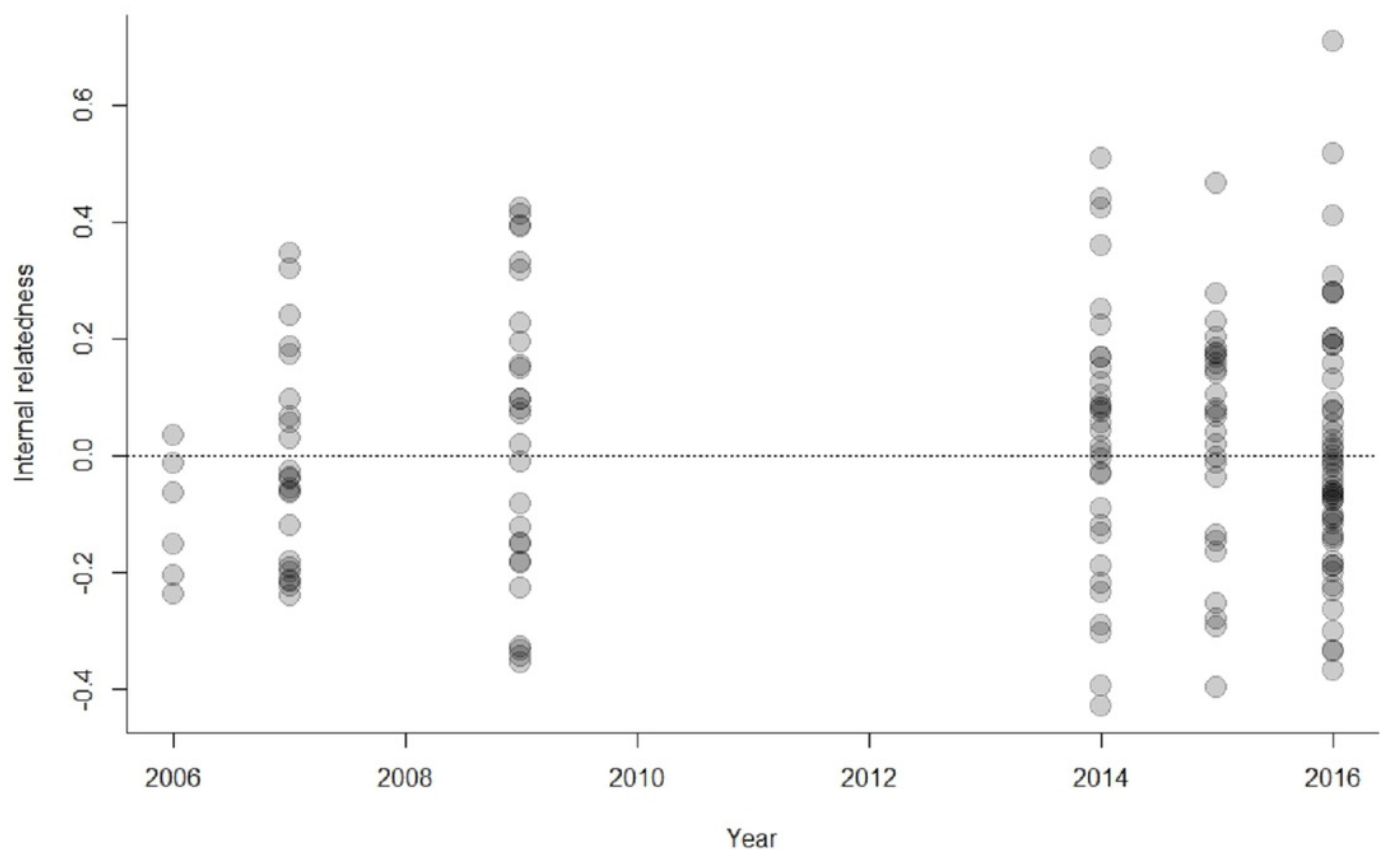
Figure 2

Effect of IR on litter size.

Marginal effect of IR based on the model shown in Table 2. The bold solid line is the fitted effect of IR on litter size at the mean of age and year in the wild population. Note that year has a strong effect on litter size, so the vertical positioning (intercept) of this line will vary for other years from the trend shown (later years having a lower intercept than earlier years; Table 2). The fine solid lines indicating the 95% CIs; raw data are overlaid.