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

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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 may be entering an extinction vortex and that to protect the population genetic rescue should be considered. 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.

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Abstract

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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

gene flow and loss of genetic diversity (relative to larger, more connected populations) as an outcome of genetic drift and inbreeding (Charlesworth & Willis 2009).

Since the discovery of DFTD in the mid-1990s, the national and international conservation community has come together and research into Tasmanian devil biology has grown rapidly, including studies of DFTD epidemiology (e.g. Hamede *et al.* 2008; McCallum *et al.* 2009; Hamede *et al.* 2012), devil behaviour (e.g. Sinn *et al.* 2014), ecological impacts (e.g. Hollings *et al.* 2014), population genetics (e.g. Lachish *et al.* 2011; Grueber *et al.* 2015; Epstein *et al.* 2016; Hendricks *et al.* 2017), *ex situ* conservation (e.g. Hogg *et al.* 2016) and translocations (e.g. Rogers *et al.* 2016; Thalmann *et al.* 2016; Wise *et al.* 2016; Grueber *et al.* 2017). As DFTD spread from the north-east across Tasmania, devil populations have been monitored by the Save the Tasmanian Devil Program (STDP) since 2004 (Lazenby *et al.* 2018). One of the last-known DFTD-free populations is located at Woolnorth (40.77° S, 144.77° E), north-west Tasmania (Farquharson *et al.* 2018; Lazenby *et al.* 2018). Since 2014, this population has suffered an extreme decline in reproductive output, the cause of which remains unclear (Farquharson *et al.* 2018). That is, between 2004 and 2009, the proportion of females breeding at Woolnorth was between 60 and 80%, however between 2014 and 2016 the proportion of females breeding was approximately 20%, a 40-60% reduction in a five-year period (Farquharson *et al*. 2018). Although for a number of carnivorous marsupials a correlation between climate and litter sizes has been shown (Fisher *et al.* 2001; Collett *et al.* 2018), this does not appear to be the sole driver of the reduction of female reproductive output in Tasmanian devils at Woolnorth (Farquharson et al 2018).

Here we aimed to test whether the observed decline in wild devil reproductive fitness (specifically litter sizes) is a result of accumulating inbreeding. Inbreeding depression occurs when an accumulation of deleterious recessive alleles lowers individual heterozygosity, negatively impacting individual fitness relative to less-inbred individuals or populations (Keller & Waller 2002; Frankham *et al.* 2017). Previous genetic research on a captive Tasmanian devil population revealed inter-individual variation in inbreeding, but no signs of inbreeding depression (Gooley *et al.* 2017). Although inbreeding depression is easier to study in controlled environments (such as captivity), it may be more consequential in the wild, as environmental conditions are more severe (Joron & Brakefield 2003; Armbruster & Reed 2005; de Boer *et al.* 2015). Thus, studies of inbreeding depression in captive environments may underestimate the impact on inbreeding on fitness in the wild (Kristensen *et al.* 2008; Gooley *et al.* 2017). In addition, wild populations that experience inbreeding depression are more vulnerable to extinction (Keller & Waller 2002), and so isolated populations may need genetic rescue to combat the effects of inbreeding (Frankham 2015; Frankham *et al.* 2017).

Here we use multilocus heterozygosity to investigate inbreeding and inbreeding depression at the DFTD-free population of devils at Woolnorth. We aimed to test: 1) whether inbreeding is occurring in the devil population at Woolnorth, and 2) whether inbreeding is associated with the observed reduction in reproduction (specifically litter sizes). The results of this study will inform the ongoing management of fragmented devil populations in the face of DFTD.

Materials & Methods

Sample collection and genotyping

Samples were collected by the STDP following their Standard Operating Procedure (see Appendix 5 in Hogg *et al.* 2019) and shared with the University of Sydney for genetic analysis. DNA samples and corresponding reproductive and demographic data were available for years 2006, 2007, 2009, 2014, 2015 and 2016. Reproductive output for females was taken as the estimated count of offspring produced (i.e. "litter size"), following Farquharson et al. (2018). Female devils are limited to a maximum of 4 offspring per breeding event (Guiler 1970). As is standard practice for documenting reproductive output in Tasmanian devils (following Keeley *et al.* 2012; Farquharson *et al.* 2018), litter size was estimated by the presence and count of pouch young for all years except 2009. The 2009 monitoring trip occurred later in the year, so litter size was estimated by the presence and count of active teats (indicating pouch young had been denned). As devils are marsupials, pouch young attach to the teat shortly after birth, and remain attached for approximately 4 months. Unoccupied teats where no pouch young attach after birth will noticeably regress (Hesterman *et al.* 2008). Denned devils (~5-10 months post birth) will continue to suckle keeping the teat active providing an indication of the number of offspring that had birthed and attached to a teat. In total, 168 wild Tasmanian devils (90 females and 78 males) were included in this study. Male reproductive output could not be examined in this study due to the open nature of the population, making pedigree reconstruction from genetic data difficult.

DNA from ear biopsy samples from the 2006, 2007 and 2009 monitoring trips had been previously extracted (Hendricks *et al.* 2017), whilst samples from 2014, 2015 and 2016 were extracted using a phenol-chloroform technique (Sanbrook *et al.* 1989) and stored at -20^{o}C. Samples were genotyped with 32 putatively neutral microsatellite markers following Gooley *et al.* (2017) and Jones *et al.* (2003). A randomly chosen set of 7% were re-genotyped to estimate genotyping error. We tested for null alleles at each locus using Micro-Checker (van Oosterhout *et al.* 2004). GenAlEx (Peakall & Smouse 2006, 2012) was used to calculate observed (H_O) and expected heterozygosity (H_E) for each locus, each year, and conduct Hardy-Weinberg exact tests.

Inbreeding and inbreeding depression

Internal relatedness (IR), a multilocus heterozygosity statistic that is expected to be positively correlated with individual inbreeding coefficient (Amos *et al.* 2001), was calculated using the function GENHET (Coulon 2010) for R (R Core Team 2019). IR incorporates allele frequencies, because there is a higher chance that rare-allele homozygosity is the result of inbred mating, relative to common-allele homozygosity (Amos *et al.* 2001). All available samples, male and female, were used to estimate allele frequencies and calculate IR, so to minimise impact of yearly allele frequency chances of calculated IR values. Across our dataset, IR was very highly correlated with other common measures of multilocus heterozygosity (such as standardised observed heterozygosity, and heterozygosity-by-loci; all absolute correlation coefficients were ≥0.94), so we focussed our main statistical analyses on IR.

We examined whether inbreeding was accumulating among individuals in the population by testing for a change in IR over time using a linear model fitted in R with year as the fixed

predictor and IR as the response (N = 168). We evaluated change in the population-level of inbreeding (F_{IS}), calculated using the package hierfstat (Goudet 2005) for R.

To interpret associations between heterozygosity and litter sizes as inbreeding depression, molecular data must reflect variation in inbreeding levels among individuals, i.e. identity disequilibrium (Szulkin *et al.* 2010). This variation was quantified with the g_2 statistic (David *et al.* 2007; Szulkin *et al.* 2010), using the package *inbreedR* (Stoffel *et al.* 2016) for R, with its precision evaluated using 1,000 Monte Carlo iterations.

We tested for inbreeding depression by determining whether IR was a predictor of female litter size using linear regression. The equations used for the regression were:

- Litter size (as a proportion of the maximum 4) ~ IR + Age + Year + Intercept
- Probability of breeding ~ IR + Age + Year + Intercept
- Litter size (as a proportion of the maximum 4) ~ IR + Age + Year + Intercept

We predicted a negative slope (i.e. increased IR is associated with decreased litter sizes). Litter size was modelled as a binomial response, where the number of events (successes) equalled the inferred litter size (based on pouch status; N = 90 females), and the number of trials equalled the maximum possible litter size of four; only one observation per female was used in the modelling (N = 36 females with evidence of producing 1 or more offspring). Along with IR (our predictor of interest), age (based on tooth wear observations, Pemberton 1990) and year were also included as continuous fixed predictors (with year = 0 for 2006). Model selection was conducted using an informatic theoretic information approach following Grueber et al (2011). We report the final model effect sizes and their 95% confidence intervals (based on 1.96 x adjusted SE), in addition to their relative importance (RI, sum of Akaike weights).

To consider the effects of individual loci, we also used an information theoretic approach whereby we ranked models with the heterozygosity of each locus coded as a 0/1 for homozygote/heterozygote (following Grueber et al. [2013]). This required us to reduce the dataset to only those individuals with complete data (to account for small numbers of missing genotypes, and thus avoid inappropriate AIC-based comparisons). The reduced dataset contains 69 females with complete genotyping data; the main effects were still evident in this subset.

Results

We found no evidence of null alleles at any of our loci, and missing data was low: >90% of individuals were successfully genotyped for >90% of loci. Genotyping error rate was 0.6%. Microsatellite diversity of Woolnorth devils was low (Table 1), and similar to observations of other wild sites and captive populations (e.g. Gooley *et al.* 2017; Storfer *et al.* 2017; Grueber *et al.* 2018). Levels of IR remained constant across the study period (linear regression: β_{Year} = 0.003 ± 0.005 SE, p = 0.546; β_0 = -5.621 ± 9.295 SE, p = 0.546, N = 168 devils, Figure 1a). The same result was obtained when using observed heterozygosity, which does not take allele frequencies into account (linear regression: β_{Year} = -0.002 ± 0.002 SE, p = 0.303; β_0 = 4.400 ± 3.896 SE, p = 0.260, N = 168 devils). Similarly, considering inbreeding at the population level in respect of Hardy-Weinberg equilibrium (F_{IS}), we also found no trend over time (Figure 1b).

We were able to assess inbreeding using our dataset as we detected statistically significant identity disequilibrium (g_2 = 0.017, SE = 0.007, p-value = 0.003), indicating that variation at our molecular markers reflects variation in the level of inbreeding among individuals.

We found evidence that inbreeding depression is occurring in the female devil population -at Woolnorth as IR had a strong negative effect on overall female litter sizes (increased homozygosity [IR] was associated with decreased fitness) (Table 2). We found little evidence of an effect of IR on propensity to breed at all (weak effect size, wide error, poor relative importance; Table 2). When examined only those females that had at least one offspring, the effect of IR on litter size was confirmed (Table 2). We therefore infer our overall results are not driven by effects of IR on breeding *per se*, but that the inbreeding depression applies primarily to litter size specifically.

Considering locus-by-locus effects of heterozygosity on litter size, we found compelling evidence that three loci (Sha3o, Sha32 and Sha013) are strong determinants of litter size. This result is inferred based on those single-locus models having substantially greater support (ΔAIC > 4) than that of the multilocus estimator, although we note that effect sizes for heterozygosity in all single-locus models were weaker than the multilocus model (Table 3). Two of the strongest-effect loci (Sh3o and Sha32) showed reduced fitness in heterozygotes relative to homozygotes (negative effect of heterozygosity; opposite to predictions under inbreeding depression), while Sha013 showed improved fitness in heterozygotes (consistent with predictions). These loci had moderate rates of heterozygosity, except for Sha32, where only five heterozygotes were observed in the reduced sample set (frequency 0.072, N = 69; Table 3). Of these five individuals, four were observed in the “early” part of the study, when reproductive rates were generally high (negative effect of Year in our modelling, Table 2, Table 3), but only two produced litters, which were small (two joeys each). The observed Sha32 data for this small sample set is therefore consistent with the negative trend in the modelling (heterozygotes produced fewer offspring than expected); more data would be required to confirm this pattern.

Three further loci (Sha040, Sha039, Sh2g) has similar levels of single-locus model support as the multilocus estimator (ΔAIC < 1); their specific effects on fitness were all positive (in line with predictions), and weak (compare the magnitudes of effect sizes in Table 3). None other single-locus models were superior to the multilocus model for explaining litter size (Table 3). We believe it is reasonable to interpret our collective single-locus results as indicating that multiple loci contribute to heterozygosity-fitness correlations in female Woolnorth devils, a result that is at least partially consistent with general (genome-wide) effects, i.e. inbreeding depression.

Discussion

Here, we show that one of the last-known DFTD-free wild populations of Tasmanian devils is experiencing inbreeding depression. Although our data did not detect an increase in inbreeding over the timescale of our study, we did show that maternal IR has a negative impact on reproductive output (litter size) in wild devils. A previous study observed a significant decline in reproduction over time at Woolnorth (Farquharson *et al.* 2018). It is unclear whether inbreeding

depression may be either partially responsible for this trend, or a worrying consequence of it. However when these observations are considered alongside the findings of the current study, we suggest that the Woolnorth population may be close to a tipping point, whereby inbreeding reduces reproductive rates (perhaps in concert with other factors), which in turn further reduces population size and exacerbates the occurrence of inbreeding and inbreeding depression. This raises the management option of genetic rescue whereby supplementation could increase the reproductive fitness of this population, which is now effectively isolated due to devil facial tumour disease causing 80% declines in adjacent devil populations (Whiteley *et al.* 2015; Lazenby *et al.* 2018).

Small populations that exist in fragmented landscapes are expected to increase in mean inbreeding levels over time (Wright *et al.* 2007; Frankham *et al.* 2017) and monitoring this process is an important element of genetic management in conservation (Fredrickson *et al.* 2007; La Haye *et al.* 2012). Interestingly, for our study, the effects of inbreeding were most influential on litter size and not on a female's propensity to breed. This result suggests inbreeding as a likely causative agent in the decline in litter size previously reported (Farquharson *et al.* 2018). Given the short time-frame of the study (2006 – 2016), our failure to detect a corresponding change in IR over time may indicate that a substantive increase in population mean inbreeding levels is yet to occur. This interpretation is not unprecedented: for example, the southernmost Swedish population of arctic fox did not show an increase in inbreeding coefficients until four years after population fragmentation that occurred in the late 1990s (Noren *et al.* 2016). In any case, the declining reproductive output seen here, and previously (Farquharson *et al.* 2018), could lead to a decrease in effective population size. As of 2018, the low reproductive output of the Woolnorth population continues (STDP, *unpublished data*). As a short-lived carnivorous marsupial species, ongoing reductions in litter sizes will more than likely impact long-term population dynamics (Fisher *et al.* 2001). If this is an accurate interpretation, the likely consequence of these processes will be an eventual increase in inbreeding, and a strengthening of its negative effects. To test this hypothesis, it will be important to continue monitoring the trajectory of demographic and genetic processes in this population, given its importance as the last DFTD-free wild population of Tasmanian devils.

Devil populations, with and without DFTD, are fragmented across the landscape, so inbreeding depression may be occurring at other sites, particularly those affected by DFTD. It would be informative to continue to quantify inbreeding depression into the future to facilitate effective management of wild populations. Evidence of inter-individual variation in inbreeding at Woolnorth (g_2 analysis) indicates that we have the molecular tools available to test for inbreeding depression; the next step is to determine whether this is also true for other sites. Our results presented here contribute to the growing body of literature that is assisting the STDP to predict the outcomes of their management strategy of augmenting small wild populations to promote gene flow (Grueber *et al.* 2018; Fox & Seddon 2019).

Conclusions

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

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Figure caption

Figure 1: Inbreeding in wild Tasmanian devils at Woolnorth (males and females) over time. Panel A shows individual-level inbreeding recorded as internal relatedness (IR); each point is an individual devil. Panel B shows population-level inbreeding (deviation from Hardy-Weinberg expectations, F_{IS}); each of the faint grey lines is a microsatellite locus, with the heavy black line illustrating the mean trend. Note: annual monitoring trips were not conducted in 2008, 2010, 2011, 2012 nor 2013.

Figure 1

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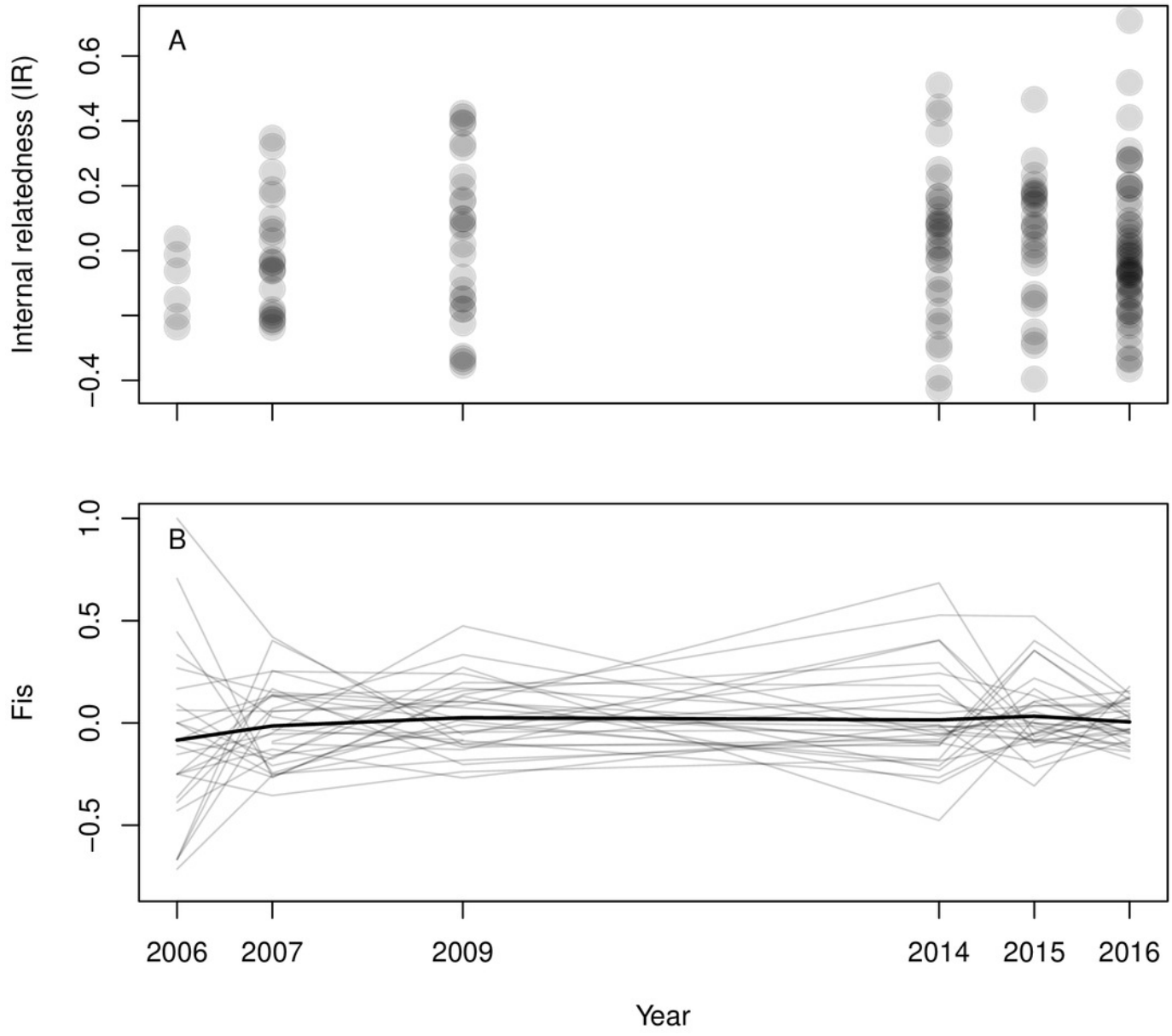
A
Internal relatedness (IR)
−0.4
0.0
0.2
0.4
0.6
B
Fis
−0.5
0.0
0.5
1.0
2006
2007
2009
2014
2015
2016
Year

Table 1(on next page)

Table 1: Genetic variation of 32 polymorphic microsatellite loci in the Woolnorth Tasmanian devil population.

Diversity is 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.

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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

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

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Table 2(on next page)

Table 2: Predictors of reproductive success in female Tasmanian devils.

Predictors have been standardised, and are the conditional average results derived from an information theoretic model selection process (see Methods).

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Model	N	Predictor	Estimate	SE	RI	R^{2*}
Litter size	90	Intercept	-1.073	0.146		0.371
		Age	0.296	0.297	0.36	
		IR	-0.953	0.275	1.00	
		Year	-2.367	0.285	1.00	
Breeding	90	Intercept	-0.489	0.262		0.311
		Age	0.605	0.556	0.38	
		IR	-0.737	0.521	0.49	
		Year	-2.527	0.561	1.00	
Litter size 1+	36	Intercept	1.304	0.228		0.312
		Age	-0.896	0.445	0.77	
		IR	-1.047	0.439	1.00	
		Year	-0.767	0.414	0.66	

Abbreviations: N = sample size, SE = adjusted standard error, IR = internal relatedness, RI = relative importance (sum of Akaike weights)

* R^2 is derived from the global model

Table 3(on next page)

Table 3: Locus-by-locus effects of heterozygosity on litter size of N = 69 female devils at Woolnorth with complete genotyping data.

Models with “Locus” IDs include a 0/1 predictor for individual heterozygosity at that locus; the “H_O” model uses multilocus observed heterozygosity, while the “Base” model excludes heterozygosity data altogether.

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			Intercept		Year		Heterozygosity		
Locus	AIC	ΔAIC.H_O[1]	Effect	SE	Effect	SE	Effect	SE	Ho[2]
Sh3o	**176.9**	**-5.5**	**1.490**	**0.300**	**-0.362**	**0.044**	**-1.178**	**0.370**	**0.377**
Sha32	**177.8**	**-4.6**	**1.422**	**0.290**	**-0.388**	**0.045**	**-1.810**	**0.623**	**0.072**
Sha013	**178.1**	**-4.3**	**0.360**	**0.351**	**-0.387**	**0.046**	**1.196**	**0.399**	**0.739**
Sha040	181.5	-0.9	0.669	0.305	-0.366	0.044	0.833	0.336	0.522
Sha039	181.9	-0.5	0.951	0.264	-0.392	0.048	0.889	0.374	0.348
Sh2g	182.0	-0.5	0.377	0.394	-0.352	0.044	0.924	0.392	0.696
H_O	182.4	-	-0.572	0.757	-0.357	0.044	4.260	1.847	0.386
Sh2p	184.0	1.6	0.584	0.360	-0.352	0.043	0.694	0.359	0.623
Sh6e	184.1	1.7	0.716	0.318	-0.354	0.043	0.639	0.330	0.522
Sh6L	185.1	2.6	0.974	0.268	-0.355	0.043	0.795	0.471	0.130
Sha023	185.3	2.8	0.857	0.293	-0.360	0.043	0.527	0.327	0.464
Sha037	185.6	3.1	0.779	0.325	-0.354	0.043	0.503	0.332	0.551
Sha001	185.7	3.3	1.148	0.263	-0.358	0.043	-1.206	0.878	0.058
Sh3a	185.8	3.4	1.250	0.286	-0.358	0.043	-0.514	0.358	0.304
Base[3]	185.9	3.5	1.090	0.257	-0.357	0.043	-	-	-
Sha024	186.3	3.9	1.177	0.269	-0.355	0.043	-0.539	0.436	0.203
Sha011	186.5	4.1	0.915	0.295	-0.351	0.043	0.397	0.334	0.348
Sh2b	186.7	4.2	0.965	0.278	-0.362	0.043	0.369	0.332	0.420
Sh2i	186.7	4.2	0.959	0.282	-0.361	0.044	0.364	0.330	0.420
Sha028	186.7	4.2	1.216	0.285	-0.359	0.043	-0.392	0.358	0.319
Sha010	186.7	4.3	1.493	0.458	-0.362	0.044	-0.459	0.421	0.812
Sha025	186.9	4.5	1.013	0.268	-0.360	0.043	0.365	0.371	0.261
Sha015	187.2	4.8	0.990	0.282	-0.362	0.044	0.274	0.330	0.493
Sha012	187.5	5.0	0.994	0.294	-0.359	0.043	0.214	0.324	0.507
Sha026	187.6	5.2	1.050	0.267	-0.360	0.043	0.194	0.362	0.304
Sha008	187.6	5.2	0.990	0.317	-0.358	0.043	0.176	0.332	0.609
Sha042	187.7	5.2	1.140	0.280	-0.356	0.043	-0.161	0.343	0.362
Sha033	187.7	5.3	1.181	0.339	-0.362	0.045	-0.143	0.338	0.391
Sh5c	187.8	5.4	1.077	0.262	-0.356	0.043	0.168	0.675	0.058
Sha014	187.9	5.5	1.104	0.293	-0.356	0.043	-0.034	0.325	0.536
Sha034	187.9	5.5	1.082	0.270	-0.356	0.043	0.036	0.417	0.174
Sha009	187.9	5.5	1.081	0.309	-0.356	0.043	0.017	0.327	0.420
Sha036	187.9	5.5	1.096	0.300	-0.357	0.045	-0.017	0.388	0.203
Sh2v	187.9	5.5	1.086	0.298	-0.357	0.043	0.009	0.323	0.493

[1] Difference in AIC between the focal model and the multilocus heterozygosity model

[2] Observed rate of heterozygosity in the sample set

[3] Sh2L was monomorphic in this subset of devils; the model is therefore equivalent to the "base" model.