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 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.

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 affected 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.* 2019). 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 in the north-east of Tasmania, the
40   national and international conservation community has come together and research into
41   Tasmanian devil biology has grown rapidly, including studies of DFTD epidemiology (e.g.
42   Hamede *et al.* 2008; McCallum *et al.* 2009; Hamede *et al.* 2012), devil behaviour (e.g. Sinn *et*
43   *al.* 2014), ecological impacts (e.g. Hollings *et al.* 2014), population genetics (e.g. Lachish *et al.*
44   2011; Grueber *et al.* 2015; Epstein *et al.* 2016; Hendricks *et al.* 2017), *ex situ* conservation (e.g.
45   Hogg *et al.* 2016) and translocations (e.g. Rogers *et al.* 2016; Thalmann *et al.* 2016; Wise *et al.*
46   2016; Grueber *et al.* 2017). As DFTD spread from the north-east across Tasmania, devil
47   populations have been monitored by the Save the Tasmanian Devil Program (STDP) since
48   2004 (Lazenby *et al.* 2018). The disease has spread in a generally south-westward direction,
49   and is now known to exist across most of the state of Tasmania, with disease-free areas limited
50   to the north-west and south-west of the state (Pemberton 2019). One of these last-known
51   DFTD-free populations is located at Woolnorth (40.77° S, 144.77° E), north-west Tasmania
52   (Farquharson *et al.* 2018; Lazenby *et al.* 2018). Since 2014, this population has suffered an
53   extreme decline in reproductive output, the cause of which remains unclear (Farquharson *et al.*
54   2018). That is, between 2004 and 2009, the proportion of females breeding at Woolnorth was
55   between 60 and 80%, however between 2014 and 2016 the proportion of females breeding was
56   approximately 20%, a 40-60% reduction in a five-year period (Farquharson *et al*. 2018).
57   Although for a number of carnivorous marsupials a correlation between climate and litter sizes
58   has been shown (Fisher *et al.* 2001; Collett *et al.* 2018), this does not appear to be the sole
59   driver of the reduction of female reproductive output in Tasmanian devils at Woolnorth
60   (Farquharson et al 2018).
61   Here we aimed to test whether the observed decline in wild devil reproductive fitness
62   (specifically litter size; devils have a maximum litter size of four pouch young; Guiler 1970) is a
63   result of accumulating inbreeding. Inbreeding depression occurs when an accumulation of
64   deleterious recessive alleles lowers individual heterozygosity, negatively impacting individual
65   fitness relative to less-inbred individuals or populations (Keller & Waller 2002; Frankham *et al.*
66   2017). Previous genetic research on a captive Tasmanian devil population revealed inter-
67   individual variation in inbreeding, but no signs of inbreeding depression (Gooley *et al.* 2017).
68   Although inbreeding depression is easier to study in controlled environments (such as captivity),
69   it may be more consequential in the wild, as environmental conditions are more severe (Joron &
70   Brakefield 2003; Armbruster & Reed 2005; de Boer *et al.* 2015). Thus, studies of inbreeding
71   depression in captive environments may underestimate the impact of inbreeding on fitness in
72   the wild (Kristensen *et al.* 2008; Gooley *et al.* 2017). In addition, wild populations that
73   experience inbreeding depression are more vulnerable to extinction (Keller & Waller 2002), and
74   so isolated populations may need genetic rescue to combat the effects of inbreeding (Frankham
75   2015; Frankham *et al.* 2017).
76   Here we use multilocus heterozygosity to investigate inbreeding and inbreeding
77   depression in the DFTD-free population of devils at Woolnorth. We aimed to test: 1) whether
78   inbreeding is occurring in the devil population at Woolnorth, and 2) whether inbreeding is
79   associated with the observed reduction in reproduction (specifically litter sizes). The results of

80 this study will inform the ongoing management of fragmented devil populations in the face of
81 DFTD.

Materials & Methods

83 *Sample collection and genotyping*

84 Samples were collected by the STDP following their Standard Operating Procedure (see
85 Appendix 5 in Hogg *et al.* 2019) and shared with the University of Sydney for genetic analysis.
86 DNA samples and corresponding reproductive and demographic data were available for years
87 2006, 2007, 2009, 2014, 2015 and 2016. Reproductive output for females was taken as the
88 estimated count of offspring produced (i.e. "litter size"), following Farquharson et al. (2018).
89 Female devils are limited to a maximum of 4 offspring per breeding event (Guiler 1970). As is
90 standard practice for documenting reproductive output in Tasmanian devils (following Keeley *et*
91 *al.* 2012; Farquharson *et al.* 2018), litter size was estimated by the presence and count of
92 pouch young for all years except 2009. The 2009 monitoring trip occurred later in the year, so
93 litter size was estimated by the presence and count of active teats (indicating pouch young had
94 been denned). As devils are marsupials, pouch young attach to the teat shortly after birth, and
95 remain attached for approximately 4 months. Unoccupied teats where no pouch young attach
96 after birth will noticeably regress (Hesterman *et al.* 2008). Denned devils (~5-10 months post
97 birth) will continue to suckle keeping the teat active providing an indication of the number of
98 offspring that had birthed and attached to a teat. In total, 168 wild Tasmanian devils (90 females
99 and 78 males) were included in this study; none provided replicate measurements. Male
100 reproductive output could not be examined in this study due to the open nature of the
101 population, making pedigree reconstruction from genetic data difficult.
102   DNA from ear biopsy samples from the 2006, 2007 and 2009 monitoring trips had been
103 previously extracted (Hendricks *et al.* 2017), whilst samples from 2014, 2015 and 2016 were
104 extracted using a phenol-chloroform technique (Sambrook *et al.* 1989) and stored at -20°C.
105 Samples were genotyped with 32 putatively neutral microsatellite markers following Gooley *et*
106 *al.* (2017) and Jones *et al.* (2003). A randomly chosen set of 7% were re-genotyped to estimate
107 genotyping error. We tested for null alleles at each locus using Micro-Checker (van Oosterhout
108 *et al.* 2004), null allele frequencies per year and per locus were calculated using the method of
109 Brookfield (1996) and tabulated via Genepop (Raymond & Rousset 1995; Rousset 2008).
110 GenAlEx (Peakall & Smouse 2006, 2012) was used to calculate observed (H_O) and expected
111 heterozygosity (H_E) for each locus, each year, and conduct Hardy-Weinberg exact tests.

112 *Inbreeding and inbreeding depression*

113 Internal relatedness (IR), a multilocus heterozygosity statistic that is expected to be positively
114 correlated with individual inbreeding coefficient (Amos *et al.* 2001), was calculated using the
115 function GENHET (Coulon 2010) for R (R Core Team 2019). IR incorporates allele frequencies,
116 because there is a higher chance that rare-allele homozygosity is the result of inbred mating,
117 relative to common-allele homozygosity (Amos *et al.* 2001). All available samples, male and
118 female, were used to estimate allele frequencies and calculate IR, so to minimise impact of
119 yearly allele frequency chances of calculated IR values. Across our dataset, IR was very highly
120 correlated with other common measures of multilocus heterozygosity (such as standardised

121   observed heterozygosity, and heterozygosity-by-loci; all absolute correlation coefficients were
122   ≥0.94), so we focussed our main statistical analyses on IR.
123   We examined whether inbreeding was accumulating among individuals in the population
124   by testing for a change in IR over time using a linear model fitted in R with year as the fixed
125   predictor and IR as the response (N = 168). We evaluated change in the population-level of
126   inbreeding (F_{IS}), calculated using the package hierfstat (Goudet 2005) for R.
127   To interpret associations between heterozygosity and litter sizes as inbreeding
128   depression, molecular data must reflect variation in inbreeding levels among individuals, i.e.
129   identity disequilibrium (Szulkin et $al.$ 2010). This variation was quantified with the g_2 statistic
130   (David et $al.$ 2007; Szulkin et $al.$ 2010), using the package $inbreedR$ (Stoffel et $al.$ 2016) for R,
131   with its precision evaluated using 1,000 Monte Carlo iterations.
132   We tested for inbreeding depression by determining whether IR was a predictor of
133   female litter size using linear regression. The equations used for the regression were:

134   • Litter size: $logit\left(\frac{litter\ size}{4}\right) = \beta_0 + \beta_1 IR_i + \beta_2 age_i + \beta_3 year_i + \varepsilon_i$

135   • Probability of breeding: $logit(P_{breed}) = \beta_0 + \beta_1 IR_i + \beta_2 age_i + \beta_3 year_i + \varepsilon_i$

136   Where β_0 is the intercept, β_{1-3} are regression coefficients associated with the specified
137   predictor variables, and ε_i is the error term. We also attempted to add year as a random fixed
138   factor (e.g. following Barr et $al.$ 2013), but only our litter size model converged. Those results
139   were qualitatively similar to our main findings, and so are presented in Supplementary Results
140   for comparison. Litter size was modelled as a binomial response using the cbind function in the
141   R $base$ package, where the number of offspring was the number of binomial "events" and the
142   number of trials was 4 (maximum possible litter size). The litter size model was fitted twice: once
143   with the litter sizes of all females (N = 90) and with only those females that showed evidence of
144   breeding (i.e. producing 1 or more offspring, N = 36). Inbreeding depression is expected to
145   produce a negative slope for IR (our predictor of interest), i.e. increased IR is associated with
146   decreased litter sizes. Age (based on tooth wear observations, Pemberton 1990) and year were
147   also included as continuous fixed predictors (with year = 0 for 2006). Model selection was
148   conducted using an information theoretic approach following Grueber et al (2011), with
149   standardisation following Gelman (Gelman 2008) using the package arm (Gelman & Su 2015),
150   and multimodel inference performed using the package MuMIn (Bartoń 2009). We report the
151   final model effect sizes and their 95% confidence intervals (based on 1.96 x adjusted SE), in
152   addition to their relative importance (RI, sum of Akaike weights), and the R^2 of the global model
153   calculated using the package rsq (Zhang 2018).
154   To consider the effects of individual loci in generating HFCs (i.e. "local effects"; see
155   Szulkin et $al.$ 2010), we tested the hypothesis that heterozygosity of some loci may be
156   individually more informative of variation in fitness than a statistic that measures the combined
157   effect of all loci. This prediction would be upheld if an overall effect of multilocus heterozygosity
158   is driven by only one or a few loci. It is important to note that, under inbreeding, heterozygosity
159   values of individual loci are not independent, and so it is the magnitudes of relative effect sizes
160   that are important (Szulkin et $al.$ 2010). The most widely-cited method for testing the local
161   effects hypothesis uses multiple regression, whereby each locus is fitted simultaneously, and
162   the slopes compared (Szulkin et $al.$ 2010). This method requires a complete dataset (no missing
163   genotype data, as incomplete cases are excluded from standard multiple regressions;

164   Nakagawa & Freckleton 2008) and, to avoid overfitting, a large sample size relative to the
165   number of loci. In linear regression, a ratio of cases:predictors of approximately 10 – 20 is
166   recommended for fitting a statistically robust regression (Harrell 2015). For our dataset, we had
167   32 loci and 69 complete cases (females with no missing genotype data), falling far short of the
168   recommended data required for this method. We therefore approached the need to model our
169   loci separately but simultaneously, and alongside multilocus heterozygosity, by using an
170   information theoretic approach. We considered the effect of a locus' heterozygosity on fitness as
171   an independent local-effect hypothesis by fitting separate models for each locus, and
172   considered all loci collectively by ranking and comparing their AIC values to draw inference
173   based on both the degree of support for each of model, and the effect sizes (slopes) of
174   heterozygosity. Our model set included:

- Single-locus models, which include observed heterozygosity of each locus fitted
  separately (coded as a 0/1 for homozygote/heterozygote; following Grueber et al.
  [2013]); 32 models in total. These models all also include informative non-genetic
  parameters (as in the "base" model, see below).
- A "base" model, which excluded heterozygosity data altogether. This model can be
  considered as our null hypothesis for the purposes of examining local effects, including
  any non-genetic parameters that were found to influence fitness in our main analysis
  (namely Year, see Results).
- An "H_O" multilocus heterozygosity model, which fits observed H_O averaged across all
  loci. The H_O statistic was used as our multilocus measure (as opposed to IR) to facilitate
  direct comparison with the single-locus models. The H_O model also includes informative
  non-genetic parameters (as in the "base" model).

187   All 34 models were quantitatively ranked and compared using AIC; our "base" and "H_O" models
188   served as reference points for calculating ΔAIC. Models with lower AIC are interpreted as
189   having stronger support, and |ΔAIC| < 2 as models with similar levels of support (Burnham &
190   Anderson 1998). We note that the reduced dataset of N = 69 females produced qualitatively the
191   same results as in our main analysis.

192   **Results**

193   Inferred null allele frequencies were very low for most loci/years (Table 1), and we had little
194   missing data: >90% of individuals were successfully genotyped for >90% of loci. Genotyping
195   error rate was 0.6%. Microsatellite diversity of Woolnorth devils was low (Table 1), and
196   similar to observations of other wild sites and captive populations (e.g. Gooley et al. 2017;
197   Storfer et al. 2017; Grueber et al. 2019). Levels of IR remained constant across the study
198   period (linear regression: β_{year} = 0.003 ± 0.005 SE, p = 0.546; β_0 = -5.621 ± 9.295 SE, p =
199   0.546, N = 168 devils, Figure 1a). The same result was obtained when using observed
200   heterozygosity, which does not take allele frequencies into account (linear regression: β_{year} =
201   -0.002 ± 0.002 SE, p = 0.303; β_0 = 4.400 ± 3.896 SE, p = 0.260, N = 168 devils). Similarly,
202   considering inbreeding at the population level in respect of Hardy-Weinberg equilibrium (F_{IS}),
203   we also found no trend over time (Figure 1b).
204   We were able to assess inbreeding using our dataset as we detected statistically
205   significant identity disequilibrium (g_2 = 0.017, SE = 0.007, p-value = 0.003), indicating that

206   variation at our molecular markers reflects variation in the level of inbreeding among
207   individuals.
208   We found evidence that inbreeding depression is occurring in the female devil population
209   at Woolnorth as IR had a strong negative effect on overall female litter sizes (increased
210   homozygosity [IR] was associated with decreased fitness) (Table 2). We found little evidence of
211   an effect of IR on propensity to breed at all (weak effect size, wide error, poor relative
212   importance; Table 2), but when examining only those females that had at least one offspring,
213   the effect of IR that was seen for overall litter size was confirmed (Table 2). We therefore infer
214   our overall results are not driven by effects of IR on breeding *per se*, but that the inbreeding
215   depression applies primarily to litter size specifically.
216   Considering locus-by-locus effects of heterozygosity on litter size, we found compelling
217   evidence that three loci (Sha3o, Sha32 and Sha013) are stronger determinants of litter size than
218   multilocus heterozygosity. This result is inferred based on those single-locus models having
219   substantially greater support than that of the multilocus estimator (ΔAIC > 4; Table 3). The
220   relative effect sizes of these loci (slope of heterozygosity) are all weaker than the multilocus
221   model (Table 3) suggesting that these findings are not consistent with strong local effects. For
222   example, two of the strongest-effect loci (Sh3o and Sha32) showed reduced fitness in
223   heterozygotes relative to homozygotes; i.e. a negative effect of heterozygosity, which is
224   opposite to predictions under inbreeding depression and opposite to the main effect of H_O.
225   (Table 3). Although Sha013 showed improved fitness in heterozygotes (consistent with
226   predictions), its effect was much weaker than seen in the multilocus model (β_{H_O}(Sha013) =
227   1.196 ± 0.399 SE, while β_{H_O}(multilocus) = 4.260 ± 1.847 SE; Table 3). For the three loci with
228   greatest evidence of an effect of an HFC, Sh3o and Sha013 had moderate rates of
229   heterozygosity, while for Sha32 only five heterozygotes were observed in the reduced sample
230   (frequency 0.072, N = 69; Table 3). Of these five Sha32 heterozygotes, four were observed in
231   the "early" part of the study, when reproductive rates were generally high (negative effect of
232   Year in our modelling, Table 2, Table 3), but only two produced litters, which were small (two
233   joeys each). The observed Sha32 data for this small sample set is therefore consistent with the
234   negative trend seen in the modelling results (heterozygotes produced fewer offspring than
235   expected); more data would be required to confirm this pattern.
236   Five further loci (Sha040, Sha039, Sh2g, Sh2p and Sh6e) have similar levels of single-
237   locus model support as the multilocus estimator (|ΔAIC| < 2); their effects on fitness were all
238   positive (in line with predictions and consistent with the multilocus predictor), and all were
239   weaker than the multilocus predictor (compare the β_{H_O} values in Table 3). No other single-locus
240   models were superior to the multilocus model for explaining litter size (Table 3). As none of the
241   slopes of our strongest single-locus effects are of greater magnitude than the main effect of
242   multilocus heterozygosity, we interpret our results as consistent with general (genome-wide)
243   effects, i.e. inbreeding depression.

Discussion

245   Here, we show that one of the last-known DFTD-free wild populations of Tasmanian devils is
246   experiencing inbreeding depression. Although our data did not detect an increase in inbreeding

247   over the timescale of our study, we did show that maternal IR has a negative impact on
248   reproductive output (litter size) in wild devils. A previous study observed a significant decline in
249   reproduction over time at Woolnorth (Farquharson *et al.* 2018). It is unclear whether inbreeding
250   depression may be either partially responsible for this trend, or a worrying consequence of it.
251   However when these past observations are considered alongside the findings of the current
252   study, we suggest that the Woolnorth population may be close to a tipping point, whereby
253   inbreeding reduces reproductive rates (perhaps in concert with other factors), which in turn
254   further reduces population size and exacerbates the occurrence of inbreeding and inbreeding
255   depression. This raises the management option of genetic rescue for Woolnorth, whereby
256   supplementation could increase the reproductive fitness of this population, which is now
257   effectively isolated due to devil facial tumour disease causing 80% declines in adjacent devil
258   populations (Whiteley *et al.* 2015; Lazenby *et al.* 2018).
259   Small populations that exist in fragmented landscapes are expected to increase in mean
260   inbreeding levels over time (Wright *et al.* 2007; Frankham *et al.* 2017) and monitoring this
261   process is an important element of genetic management in conservation (Fredrickson *et al.*
262   2007; La Haye *et al.* 2012). Interestingly, for our study, the effects of inbreeding were most
263   influential on litter size and not on a female's propensity to breed. This result suggests
264   inbreeding as a likely causative agent in the decline in litter size previously reported
265   (Farquharson *et al.* 2018). Given the short time-frame of the study (2006 – 2016), our failure to
266   detect a corresponding change in IR over time may indicate that a substantive increase in
267   population mean inbreeding levels is yet to occur. This interpretation is not unprecedented: for
268   example, the southernmost Swedish population of arctic fox did not show an increase in
269   inbreeding coefficients until four years after population fragmentation that occurred in the late
270   1990s (Noren *et al.* 2016). In any case, the declining reproductive output seen here, and
271   previously (Farquharson *et al.* 2018), could lead to a decrease in effective population size. As of
272   2018, the low reproductive output of the Woolnorth population continues (STDP, *unpublished*
273   *data*). As a short-lived carnivorous marsupial species, ongoing reductions in litter sizes will more
274   than likely impact long-term population dynamics (Fisher *et al.* 2001). If this is an accurate
275   interpretation, the likely consequence of these processes will be an eventual increase in
276   inbreeding, and a strengthening of its negative effects. To test this hypothesis, it will be
277   important to continue monitoring the trajectory of demographic and genetic processes in this
278   population, given its importance as the last DFTD-free wild population of Tasmanian devils.
279   Devil populations, with and without DFTD, are fragmented across the landscape, so
280   inbreeding depression may be occurring at other sites, particularly those affected by DFTD. It
281   would be informative to continue to quantify inbreeding depression into the future to facilitate
282   effective management of wild populations. Evidence of inter-individual variation in inbreeding at
283   Woolnorth (g_2 analysis) indicates that we have the molecular tools available to test for
284   inbreeding depression; the next step is to determine whether this is also true for other sites. Our
285   results presented here contribute to the growing body of literature that is assisting the STDP to
286   predict the outcomes of their management strategy of augmenting small wild populations to
287   promote gene flow (Fox & Seddon 2019; Grueber *et al.* 2019).
288   Extensive modelling will be informative for predicting the long-term consequences of
289   reduced reproductive output on devil population dynamics and growth. At sites where DFTD is
290   present, there have been observed increases in precocial breeding (1 year olds breeding) and

291   increased litter sizes, highlighting the complex interplay between reproductive parameters and
292   population sustainability in the presence of DFTD (Jones *et al.* 2008; Grueber *et al.* 2018;
293   Lazenby *et al.* 2018). However, the degree to which this population compensation permits long-
294   term population growth is still unclear (Lazenby et al 2018), and it is similarly unclear whether
295   similar processes will occur in the face of inbreeding depression. Previous modelling to assess
296   the retention of rare alleles at DFTD-present sites when population sizes are small showed that
297   population supplementation would be required to ensure long-term genetic viability (Grueber et
298   al 2019). To predict the long-term consequences of the inbreeding depression observed here, a
299   more comprehensive modelling exercise is required. These models need to account for possible
300   trade-offs and interactions among inbreeding, reproductive dynamics, changes in survivorship in
301   the presence of DFTD, and other ecological parameters such as the impact of drought/changing
302   climate, roadkill, habitat fragmentation etc. We also acknowledge that translocations carry risks,
303   which are incorporated into management planning when determining the cost/benefit trade-off of
304   supplementing wild populations (Ewen *et al.* 2012). For example, in addition to common
305   concerns such as survival rates (e.g. Thalmann *et al.* 2016), the Save the Tasmanian Devil
306   Program also considers the potential for vehicle strike (e.g. Grueber *et al.* 2017) and other
307   behavioural factors (e.g. Sinn *et al.* 2014), microbiome changes (e.g. Chong *et al.* 2019) and
308   variation in the genetic contributions of translocated individuals (e.g. McLennan *et al.* 2018).
309   Consideration of the genetic impacts of translocation is critical for ensuring the long-term
310   persistence of managed populations (Weeks *et al.* 2011). For wild devils, it would be valuable to
311   consider the interplay between the apparent costs of inbreeding depression (this study) and
312   genetic diversity loss (e.g. Grueber *et al.* 2019) in an analysis that also incorporates the effects
313   of DFTD on demography (e.g. Jones *et al.* 2008; Grueber *et al.* 2018; Lazenby *et al.* 2018) and
314   genetic structure (e.g. Lachish *et al.* 2011; Epstein *et al.* 2016).

315   Conclusions

316   We have documented the first evidence of inbreeding depression in a wild population of
317   Tasmanian devils. Whether inbreeding is the driver of the observed reproductive decline at
318   Woolnorth, and/or whether the reproductive decline is driving an increase in inbreeding cannot
319   be specifically determined. Although the long-term impact of this reduced productivity on
320   population growth is unknown at this time, our data do show that inbreeding is detrimental to
321   reproductive output in this population, and has the potential to become more prevalent.
322   Augmenting this population with genetic material from other locations across Tasmania may
323   alleviate the effects of future inbreeding and minimise the occurrence of inbreeding depression.
324   Ongoing monitoring after augmentation will provide valuable insights to the impacts of
325   supplementation on population growth and inbreeding.

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513

514 **Figure caption**

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

Figure 1

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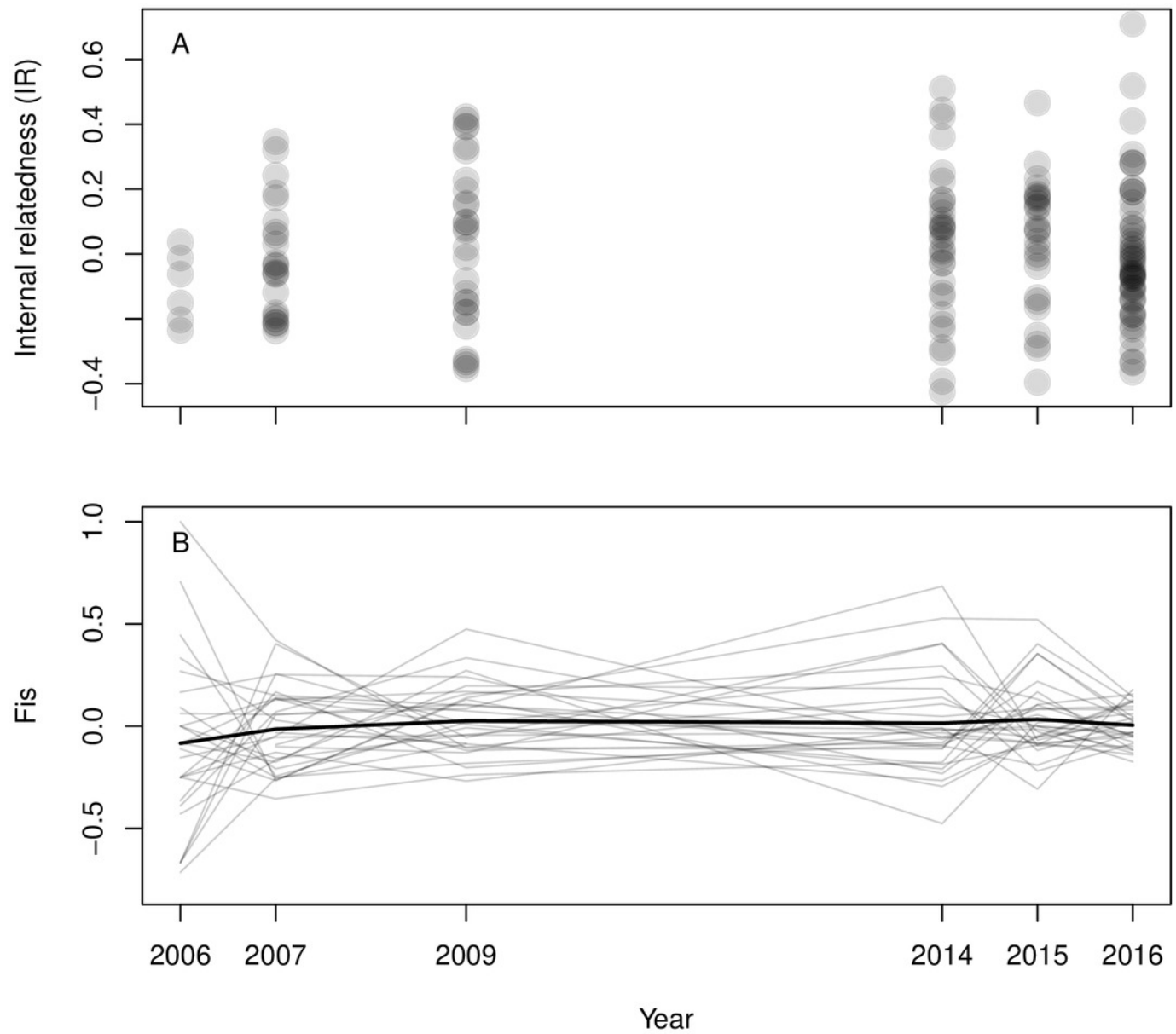
Table 1: Genetic variation of 32 polymorphic microsatellite loci in the Woolnorth Tasmanian devil population.

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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3 (H_O), unbiased estimate of expected heterozygosity (H_E) and Hardy-Weinberg Exact test (p-
4 value). Total number of devils N = 168.

| Locus[2] | N | Na | H_O | H_E | p-value | Estimated null allele frequencies per year[1] |
						2006	2007	2009	2014	2015	2016
Sh2b	147	2	0.340	0.378	0.239	0.000	0.010	0.015	0.692	0.333	0.008
Sh2g	167	3	0.701	0.646	0.053	0.000	0.000	0.000	0.000	0.000	0.100
Sh2i	168	3	0.411	0.406	0.443	0.000	0.000	0.000	0.000	0.000	0.043
Sh2p	168	3	0.667	0.617	0.300	0.000	0.000	0.000	0.046	0.061	0.042
Sh2v	168	6	0.548	0.587	0.738	0.069	0.022	0.000	0.000	0.000	0.030
Sh3a	155	3	0.226	0.245	0.078	NA	0.000	0.000	0.551	0.230	0.026
Sh3o	168	4	0.464	0.522	0.129	0.027	0.000	0.054	0.000	0.006	0.082
Sh5c	160	3	0.069	0.067	0.977	0.000	NA	0.000	0.468	0.186	0.000
Sh6e	168	2	0.435	0.412	0.452	0.629	0.404	0.558	0.415	0.607	0.555
Sh6L	167	2	0.138	0.139	0.943	0.000	0.000	0.056	0.156	0.000	0.000
Sha001	164	3	0.085	0.083	0.955	0.577	0.264	NA	0.000	0.000	0.000
Sha008	161	3	0.547	0.534	0.769	0.077	0.003	0.097	0.265	0.179	0.000
Sha009	163	4	0.319	0.297	0.954	0.516	0.199	0.000	0.145	0.019	0.000
Sha010	161	7	0.826	0.778	0.757	0.163	0.000	0.000	0.230	0.134	0.000
Sha011	167	2	0.329	0.386	0.061	0.000	0.010	0.024	0.000	0.126	0.114
Sha012	156	3	0.487	0.538	0.000	0.000	0.016	0.191	0.307	0.331	0.024
Sha013	162	7	0.710	0.675	0.718	0.000	0.058	0.000	0.200	0.164	0.000
Sha014	165	4	0.491	0.525	0.108	0.000	0.073	0.000	0.217	0.140	0.000
Sha015	155	2	0.471	0.471	0.978	0.000	0.114	0.000	0.542	0.176	0.000
Sha023	156	5	0.436	0.423	0.998	0.000	0.036	0.017	0.518	0.096	0.000
Sha024	148	2	0.209	0.199	0.486	0.000	0.000	0.173	0.603	0.286	0.160
Sha025	166	2	0.193	0.231	0.037	0.217	0.107	0.000	0.139	0.194	0.006
Sha026	164	3	0.226	0.233	0.667	NA	0.026	0.000	0.261	0.179	0.004
Sha028	148	5	0.264	0.241	0.970	0.000	0.000	0.000	0.647	0.374	0.000
Sha032	147	3	0.061	0.060	0.986	NA	0.000	0.000	0.707	0.419	NA
Sha033	166	2	0.331	0.301	0.178	0.000	0.104	0.000	0.121	0.049	0.003
Sha034	166	3	0.193	0.200	0.580	NA	0.000	0.021	0.175	0.000	0.117
Sha036	165	2	0.248	0.295	0.048	0.000	0.000	0.024	0.261	0.094	0.122
Sha037	164	6	0.610	0.688	0.000	0.000	0.045	0.067	0.245	0.026	0.034
Sha039	160	4	0.400	0.407	0.961	0.000	0.026	0.118	0.252	0.221	0.082
Sha040	165	5	0.612	0.599	0.000	0.000	0.000	0.171	0.048	0.124	0.019
Sha042	163	2	0.313	0.297	0.479	0.000	0.149	0.033	0.217	0.122	0.095

5 [1] Estimated using the method of Brookfield (1996); NA indicates a locus that was monomorphic
6 in the specified year dataset.
7 [2] The ten "Sh" markers were developed by Jones *et al.* (2003); the remaining 22 "Sha" markers
8 were developed by Gooley *et al.* (2017)

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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).

1 **Table 2:** Predictors of reproductive success in female Tasmanian devils. Predictors have been
2 standardised, and are the conditional average results derived from an information theoretic
3 model selection process (see Methods); full model sets are provided in Supplementary Table
4 S1.

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	

6 Abbreviations: N = sample size, SE = adjusted standard error, IR = internal relatedness, RI = relative
7 importance (sum of Akaike weights)
8 * R^2 is derived from the global model

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.

1 **Table 3**: Locus-by-locus effects of heterozygosity on litter size of N = 69 female devils at
2 Woolnorth with complete genotyping data. Models with "Locus" IDs include a 0/1 predictor for
3 individual heterozygosity at that locus; the "H_O" model uses multilocus observed
4 heterozygosity, while the "Base" model excludes heterozygosity data altogether.

Locus	AIC	ΔAIC_{base}[1]	ΔAIC_{H_O}[2]	β_0	$SE(\beta_0)$	β_{year}	$SE(\beta_{year})$	β_{H_O}	$SE(\beta_{H_O})$	H_O[3]
Sh3o	**176.9**	**-9.0**	**-5.5**	**1.490**	**0.300**	**-0.362**	**0.044**	**-1.178**	**0.370**	**0.377**
Sha32	**177.8**	**-8.1**	**-4.6**	**1.422**	**0.290**	**-0.388**	**0.045**	**-1.810**	**0.623**	**0.072**
Sha013	**178.1**	**-7.8**	**-4.3**	**0.360**	**0.351**	**-0.387**	**0.046**	**1.196**	**0.399**	**0.739**
Sha040	181.5	**-4.4**	-0.9	0.669	0.305	-0.366	0.044	0.833	0.336	0.522
Sha039	181.9	**-4.0**	-0.5	0.951	0.264	-0.392	0.048	0.889	0.374	0.348
Sh2g	182.0	**-3.9**	-0.5	0.377	0.394	-0.352	0.044	0.924	0.392	0.696
H_O	182.4	**-3.5**	-	-0.572	0.757	-0.357	0.044	4.260	1.847	0.386
Sh2p	184.0	-1.9	1.6	0.584	0.360	-0.352	0.043	0.694	0.359	0.623
Sh6e	184.1	-1.8	1.7	0.716	0.318	-0.354	0.043	0.639	0.330	0.522
Sh6L	185.1	-0.8	2.6	0.974	0.268	-0.355	0.043	0.795	0.471	0.130
Sha023	185.3	-0.6	2.8	0.857	0.293	-0.360	0.043	0.527	0.327	0.464
Sha037	185.6	-0.3	3.1	0.779	0.325	-0.354	0.043	0.503	0.332	0.551
Sha001	185.7	-0.2	3.3	1.148	0.263	-0.358	0.043	-1.206	0.878	0.058
Sh3a	185.8	-0.1	3.4	1.250	0.286	-0.358	0.043	-0.514	0.358	0.304
Base[4]	185.9	-	3.5	1.090	0.257	-0.357	0.043	-	-	-
Sha024	186.3	0.4	3.9	1.177	0.269	-0.355	0.043	-0.539	0.436	0.203
Sha011	186.5	0.6	4.1	0.915	0.295	-0.351	0.043	0.397	0.334	0.348
Sh2b	186.7	0.8	4.2	0.965	0.278	-0.362	0.043	0.369	0.332	0.420
Sh2i	186.7	0.8	4.2	0.959	0.282	-0.361	0.044	0.364	0.330	0.420
Sha028	186.7	0.8	4.2	1.216	0.285	-0.359	0.043	-0.392	0.358	0.319
Sha010	186.7	0.8	4.3	1.493	0.458	-0.362	0.044	-0.459	0.421	0.812
Sha025	186.9	1.0	4.5	1.013	0.268	-0.360	0.043	0.365	0.371	0.261
Sha015	187.2	1.3	4.8	0.990	0.282	-0.362	0.044	0.274	0.330	0.493
Sha012	187.5	1.6	5.0	0.994	0.294	-0.359	0.043	0.214	0.324	0.507
Sha026	187.6	1.7	5.2	1.050	0.267	-0.360	0.043	0.194	0.362	0.304
Sha008	187.6	1.7	5.2	0.990	0.317	-0.358	0.043	0.176	0.332	0.609
Sha042	187.7	1.8	5.2	1.140	0.280	-0.356	0.043	-0.161	0.343	0.362
Sha033	187.7	1.8	5.3	1.181	0.339	-0.362	0.045	-0.143	0.338	0.391
Sh5c	187.8	1.9	5.4	1.077	0.262	-0.356	0.043	0.168	0.675	0.058
Sha014	187.9	2.0	5.5	1.104	0.293	-0.356	0.043	-0.034	0.325	0.536
Sha034	187.9	2.0	5.5	1.082	0.270	-0.356	0.043	0.036	0.417	0.174
Sha009	187.9	2.0	5.5	1.081	0.309	-0.356	0.043	0.017	0.327	0.420
Sha036	187.9	2.0	5.5	1.096	0.300	-0.357	0.045	-0.017	0.388	0.203
Sh2v	187.9	2.0	5.5	1.086	0.298	-0.357	0.043	0.009	0.323	0.493

5 [1] Difference in AIC between the focal model and the "base" model, which excludes genetic data
6 [2] Difference in AIC between the focal model and the multilocus heterozygosity model
7 [3] Observed rate of heterozygosity in the sample set, for the specified locus
8 [4] Sh2L was monomorphic in this subset of devils; the model is therefore excluded from the table, as it
9 is identical to the "base" model.