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Research article

A retrospective examination of factors associated with breeding success of Tasmanian devils in captivity (2006 to 2012)

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Abstract

The captive Tasmanian devil *Sarcophilus harrisii* insurance population was initiated in 2005 in response to rapid wild population declines due to a fatal, transmissible cancer. Wild-caught founders were sourced at the age of dispersal from disease-free areas during the programme's early years. Despite devils having a long history of captive breeding, breeding rates remain low. Studbook records, annual reports and breeding recommendations in 2006–2012 were evaluated to identify factors influencing reproductive success in the breeding programme. During the study period, 39% of females (range 26–49%) paired for breeding successfully produced young. Captive-born and wild-caught females produced young at a similar rate (38.2% versus 42.6%). On average females produced their first litters at 2.1 years old, the average age of sexual maturity. The likelihood of successful production of young declined as the female increased in age (2 years=46%; 3 years=40.8%, 4 years=23.2%). Litter size did not differ between wild-caught or captive-born females (2.8±1.1 versus 2.6±1.1 respectively) and was unaffected by dam age. Females were more likely to be successful if paired with an older male. Despite adequate breeding opportunities, 27.4% (26/95) of wild female founders failed to reproduce and most successful female founders only ever produced a single litter (41/69; 59.4%). By the end of 2012, 53.7% of female (51/95) and 80.2% of male (57/71) founder lines were still active, confirming a founder line loss of 34.9%. While data were limited, enclosure features such as provision of dens with dirt flooring may influence breeding success. Although other elusive factors need to be identified, there is evidence that female devils need to be provided breeding opportunities at two years old and paired with older males to maximise their overall potential for breeding success.

Introduction

The Tasmanian devil *Sarcophilus harrisii* was listed as Endangered in 2008 due to the ravages of the transmissible cancer, devil facial tumour disease. The population is currently listed as decreasing (IUCN 2020). Due to the severity and swiftness with which the transmissible cancer was decimating the wild population (Hawkins et al. 2006; Pyecroft et al. 2007), an insurance programme was initiated in 2005, in partnership between the Australasian Zoo and Aquarium Association, Save the Tasmanian Devil Program and Tasmanian Department of Primary Industries, Parks, Water and the Environment. The programme aims to maintain 95% of wild Tasmanian devil genetic diversity within the captive population for 50 years (CBSG 2008). Currently, the intensively managed breeding component of the insurance population is maintained in over a dozen institutions in six Australian states (Tasmania, New South Wales, Queensland, Victoria, South Australia and Western Australia), one territory (the Australian Capital Territory) and a number of overseas zoos (Srb 2015).

In threatened species recovery, captive breeding is one strategy used to stave off extinction. To initiate a captive breeding programme, ideally, unrelated healthy founding animals are required. Founders are often wild-caught individuals; when brought in from the wild, there is an assumption that individuals are unrelated (Lacy 1989). However, unless founders can be

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sourced from geographically isolated populations, this assumption may not be met. This new population is then managed through a studbook where all individuals, their origins, genealogy, sex, age, identification numbers, breeding history and other life history traits are kept. Using the studbook and breeding records, mean kinship, an inbreeding coefficient, is calculated and informs decisions as to which animals are to be paired for breeding with an aim to maintain adequate genetic diversity over time (Ballou and Lacy 1995). Genetic diversity can be lost through genetic drift and inbreeding depression (Ballou and Lacy 1995), so supplementing captive populations with new founders can be advantageous. Both overrepresentation and underrepresentation of founders can lead to a loss of heterozygosity, increasing inbreeding and decreasing breeding success (Lacy 1989), which combined with other challenges may impede the viability of a captive breeding programme. Due to inadequate breeding success as a result of failed pairings, lack of animal transfers or other factors, efforts to evaluate breeding and transfer recommendations, breeding outcomes and studbook data have increased in a quest to improve captive breeding programmes in the long term (Bauman et al. 2019; Che-Castaldo et al. 2019).

Breeding endangered species is a complex process. Many species have a history of suboptimal captive breeding success e.g. cheetahs *Acinonyx jubatus* (Penfold et al. 2014), eastern black rhinoceros *Diceros bicornis michaeli* (Edwards et al. 2015) and eastern barred bandicoot *Perameles gunnii* (Hartnett et al. 2018), exacerbating existing conservation challenges. Reproductive failure can be multifactorial with contributing factors such as stress, lack of or inappropriate mate choice, enclosure design and size, social structure, age at first introduction to a potential mate, insufficient breeding recommendations, inconsistent consideration of a species' reproductive biology and reproductive behaviour, and prior experience (Hartnett et al. 2018; Penfold et al. 2014; Wachter et al. 2011; Wingfield and Sapolsky 2003).

Tasmanian devils have been held and bred in captivity for over a century, but historically breeding efforts have been variable, likely due to the then abundance of the species and a lack of need to breed them (Fleay 1935; Kelly 1993; Roberts 1915). Prior to development of the insurance population, there was an Australasian Species Management Program for the Tasmanian devil. However, breeding and transfer recommendations were often not followed, likely due to the ability to source additional wild animals if needed (Zoo and Aquarium Association, personal communication). Originally thought to be mono-oestrous, female devils are facultative poly-oestrous with the potential of undergoing up to three oestrous cycles within a breeding season (typically February/March to May/June) if pouch young (PY) are not successfully produced or are lost at or shortly after birth (Guiler 1970; Hesterman et al. 2008; Keeley et al. 2012a). Devils have an extended oestrus of 6–10 days during which mating occurs prior to a presumed period of sperm storage and 12.5 day gestation, and therefore give birth approximately three weeks after mating (Keeley et al. 2012a). Female devils are generally known to breed between the ages of two and four years with reproductive senescence typically occurring by the age of five. They can produce a single litter of up to four young each year (Keeley et al. 2012a; Kelly 1993). Female devils have been recorded breeding in the wild and in captivity as young as 1 year old, but this appears to be restricted to a small number of individuals that become precocial breeders perhaps through attainment of a critical body weight range (Lachish et al. 2009). As devils are a solitary species, coming together only for breeding or carcass feeding, adult devils are typically housed individually and only brought together for breeding when signs of oestrus, such as inappetite, are observed in females (Guiler 1970; Keeley et al. 2012a; Kelly 2007; Pemberton and Renouf 1993).

Although several record-based studies over the last decade (Farquharson et al. 2017, 2018; Hogg et al. 2015) have attempted to identify factors associated with suboptimal breeding success in the Tasmanian devil insurance population, elusive factors inhibiting significant improvement seem to remain. The aim of this study was to evaluate potential factors associated with reproductive success in the Tasmanian devil captive breeding programme. Specifically this study includes 1) evaluation of historic records for the first seven years (2006–2012) of the insurance programme to examine changes in breeding rates and litter size over time; 2) examination of the potential effects of birth origin (wild-caught versus captive-born) and age on female reproductive success and litter size; and 3) evaluation of pen size and proximity of conspecifics between successful and unsuccessful females at four institutions to compare potential factors associated with reproductive success. Information provided in this study will contribute to future optimisation of Tasmanian devil captive management and breeding programmes.

Methods

Insurance population establishment

The Tasmanian devil insurance population (TDIP) was established predominately through three intakes of wild-born Tasmanian devils (2005–2008; n=122 individuals) from cancer-free areas. It was then supplemented by a few Australasian Species Management Program devils deemed appropriate for inclusion and wild devils brought into captivity opportunistically (typically PY hand-reared following euthanasia of the mother due to cancer; Hogg et al. 2015).

Insurance population breeding success 2006–2012

To evaluate the success of the TDIP during the first seven years of the programme, data regarding annual breeding recommendations and outcomes were gathered from the Tasmanian Devil Annual Reports and Recommendations published between 2006 and 2012 by the Zoo and Aquarium Association for all institutions and three purpose-built quarantine facilities (first year of the programme only). Data evaluation was restricted to intensely managed facilities as data on free-ranged enclosures and managed environmental enclosures were limited and parentage (typically sire but sometimes dam) of a large number of offspring are currently unknown. Free-ranged and managed environmental enclosures are large semi-free range fenced enclosures housing groups of male and female devils and are therefore managed for breeding on a group, not individual, level. Data from annual reports included the devil's studbook number, name, sex, breeding recommendations, pairings for breeding, breeding success, litter size, litter sex ratio and institution. This was confirmed by crosschecking against information in the Tasmanian devil studbook. Additional information including date of birth, parentage and date of death (for founder evaluation) was taken from the Tasmanian devil studbook (Srb 2015) which contains records of all current and past captive-housed or captive-born devils.

Initially, the TDIP consisted of zoological institutions on the mainland of Australia (n=11 institutions). The devils housed in managed enclosures at two facilities in Tasmania operated by the Tasmanian Department of Primary Industries, Parks, Water and the Environment were added to the breeding recommendations in 2011, and two privately owned wildlife parks in Tasmania were included in 2012. By the end of 2012, the insurance population had grown to 515 devils (75% in intensively managed facilities; Zoo and Aquarium Association 2012). With growth of the insurance population and due to space limitations, after 2012 breeding recommendations were no longer provided to all adult female devils. An increase in multiple males paired with each female meant paternity was not always known and therefore records after 2012 were not evaluated in this study. For female devils to be confirmed to have had a successful attempted pairing, the records must confirm the successful introduction of one or more males sequentially during the presumed oestrus period for 1–3 oestrous cycles. As the records rarely confirmed if the female was paired during one or more oestrous cycles, data are provided on a yearly, not cycle level.

To evaluate factors influencing female reproductive potential, a female's age, origin (wild versus captive-born), previous pairing and PY production history, and location (institution) were evaluated. For females that produced litters, the size and sex ratio of the litter were compared against her age, origin and location (institution) to determine if these factors influence litter dynamics.

To evaluate the potential for male fertility to influence female devil reproductive success, pairing of unsuccessful females with proven or unproven males and the age difference between a paired female and male devil were evaluated. A proven male was defined as one that successfully produced viable PY. An unproven male was one that was offered mating opportunities but without any confirmed pregnancies (no viable PY). If records indicated that more than one male could be paired with the female and there was no confirmation whether all suggested males were trialled, these were designated as proven (if both males had produced viable PY), unproven (if neither male had produced viable PY) or unknown (if there was both a proven and unproven male breeding recommendation and no confirmation if one or both were tried).

To evaluate founder animal success, a 'founder' was defined as an individual that was wild-born with unknown wild parents that either was brought into captivity with PY or had at least one opportunity to breed in captivity between the ages of two and four years for females or two and six for males. Most wild devils brought into the programme were caught at age of dispersal (9–14 months; Hogg et al. 2015) and others were aged using tooth wear (Jones et al. 2008). Devils that were brought into captivity but never had an opportunity to be paired for mating were excluded from the founder definition. Potential female and male founders that were housed in unmanaged free-ranged or managed environmental enclosures were excluded from analysis due to the difficulty of ascertaining whether or not they bred due to a large number of offspring with unknown or unconfirmed parentage. Founder lines were traced for both males and females to determine how many remained active or had terminated. To be considered 'active' a founder line had to have living descendants under the age of four years for a female or six for a male with the potential for breeding recommendations in subsequent years.

Housing

In mid-2011, at breeding season completion, a voluntary survey was sent to nine mainland Australian institutions that actively participated in the captive breeding programme. The survey requested institutional information on the layout, size (area, height of walls), construction material and composition of inclusions (e.g. den boxes, climbing structures) of enclosures used to house breeding Tasmanian devils during the early stages of the insurance population captive breeding programme. The survey requested information regarding the location of occupancy of breeding females and all devils during the 2011 breeding season with a request for additional years (2008 to 2010) if available. Returned data were used to extend the analysis of 'location' to include specific details on enclosure structure and features to evaluate potential environmental effects on breeding success.

Statistical analysis and data evaluation

All statistical analyses were performed using GenStat 16th edition (VSN International Ltd., UK). A linear mixed model was used to test the following fixed factors on the response variables of breeding success (production of PY) and number of PY (litter size): origin of birth (wild-caught or captive-born; for both males and females), female age and age difference between paired male and female. An analysis of variance was used to test the effects of origin of dam birth on PY sex ratio and the balance between male and female PY in litters. Location (institution) and data from individual female devils across multiple years were assumed to be independent random variables for each year and evaluated as random effects. Annual reports rarely confirmed if a female devil had one, two or three oestrous cycles in a given year (if unsuccessful) and therefore it was not possible to evaluate any potential effect of cycle number on pairing outcome nor to confirm if mating was attempted only on a single oestrous cycle or for multiple cycles per year. Therefore, unsuccessful females may have been paired one to three times within that season, with all pairings unsuccessful. Males and females were only paired when the female displayed signs of oestrus (e.g. inappetite) and were removed from each other if signs of aggression developed (Keeley et al. 2012a). 'Successful breeding' was defined as confirmation of PY at the first pouch check which occurred 2 weeks to 2 months after expected parturition. As such, a 'negative pouch check' does not rule out the possibility that the female did become pregnant but lost the PY at or shortly after birth, but is still defined as 'unsuccessful'. $P<0.05$ was considered to be significant. Data are presented as ±standard error of the mean, unless otherwise noted.

Results

Insurance population breeding success 2006–2012

For each year of the study period, there were a few discrepancies between the information reported in the annual reports and the studbook (e.g. number of PY in a litter, which females did or did not produce viable PY, paternity of PY). In all cases the information in the studbook was used as it more likely represented the most up to date information. Breeding success of the captive insurance population between 2006 and 2012 was variable but low overall (Table 1, Figure 1). Breeding success rates reported for the free-ranged and managed environmental enclosures in the 2011 and 2012 annual reports (Zoo and Aquarium Association 2012) were also low overall (58.1% and 44.2% respectively). In both 2008 and 2009 a female devil with a breeding recommendation died before being paired with a male, therefore these two animals were excluded from the dataset. A high proportion (83–100%) of annual breeding recommendations made by the Zoo and Aquarium Association were attempted (Table 1). Of the 243 breeding recommendations examined in 2006–2012, 144 (59.3%) did not result in confirmed PY production (as of the first pouch check) and 99 litters (40.7% of recommendations) were produced. Average number of PY produced per breeding female per year varied from 2.3 to 3.0 (Table 1). Captive-born females produced PY after pairing at a similar rate to wild-caught females (38.2% versus 42.6%; 39/102 versus 60/141 respectively, $P>0.05$). The average PY litter size between wild-caught and captive-born females (Table 2) was not significantly different ($P=0.25$) nor was it different if the sire was wild-caught or captive-born ($P=0.39$). Female age (2, 3 or 4 years) did not affect average PY litter size ($P=0.55$, Table 2). A total of 273 PY were produced including 139 males, 124 females and 10 of unknown sex (lost from the pouch before sex determination) with average litter size of 2.8±1.1. This resulted in an overall sex ratio of 139:124 or 52.5% male and 46.8% female offspring. The number of males and females in each litter was similar ($P>0.05$) with an overall average of 1.8±0.8 males and 1.6±0.8 females per litter. Of the single PY litters, 1 had a PY of unknown sex (did not survive until the second pouch check), 8 were male PY and 8 were female PY (one of which had reduced from 4 PY to 1 PY between

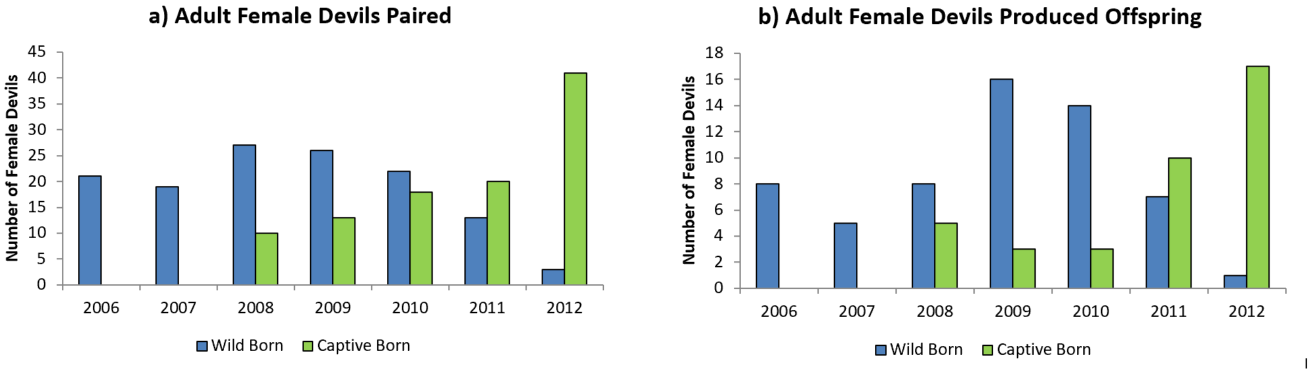

Figure 1. Total number of adult female devils that were a) paired per year and b) produced a litter of 1–4 offspring in 2006–2012, demonstrating a shift over time from breeding wild-caught founders to captive-born descendants.

the first and second pouch check). Of the multi-PY litters, most were multi-sex litters (71%; 58/82 multi-sex litters or 59% of all litters) with only 12 litters with females only (2–4 females) and 12 litters with males only (2–4 males). There was no difference in sex ratio of litters between wild-caught and captive-born females, nor among age classes (P>0.05).

Through evaluation of annual reports and studbook records, eight cases of PY loss between the first and second pouch check were identified (2008: 1 litter of 4 PY reduced to 3 PY and 1 litter of 3 PY reduced to 2 PY; 2009: 1 litter of 3 PY reduced to 2 PY; 2010: 2 litters of 4 PY reduced to 1 and 2 PY; 2011: 1 litter of 2 PY reduced to 0; 2012: 1 litter of 1 PY reduced to 0 and 1 litter of 4 PY reduced to 3 PY). These females were housed at four different institutions, were both captive- (n=5) and wild- (n=3) born and were predominately 2 years old (n=6) at the time of breeding (3 years n=1, 4 years n=1). Additional partial or full litter PY losses could have been undetected if they occurred between birth and first pouch check (generally 2 weeks after estimated birth).

Effect of age on production of young

Females with negative pouch checks after mating or pairing were on average significantly older than females with positive pouch checks (2.9±0.1 years versus 2.6±0.1 years; P=0.01). Successful litter production declined as female age increased (2 years=46%,

Table 1. Overall summary of captive Tasmanian devil insurance population (TDIP) breeding success (excluding individuals housed in free-ranged enclosures) in 2006–2012. Attempted pairings included the introduction of one or more males sequentially during the presumed oestrus period for at least one oestrous cycle. Total number of pouch young per year includes the number of young present at the first pouch check (2 weeks to 2 months post parturition). Not all institutions were provided with breeding recommendations in every year of participation. * Purpose built, temporary holding facilities in Tasmania.

Year	Number of institutions with TDIP breeding recommendations	Number of breeding pair recommendations made (females)	Number of pairings attempted	Recommendations attempted (%)	Number of successful pairings	Recommendations successful (%)	Total number of pouch young	Average pouch young per female
2006	3*	21	21	100	8	38	18	2.3
2007	4	19	19	100	5	26	13	2.6
2008	8	38	37	97	13	34	33	2.5
2009	7	38	37	97	19	50	57	3.0
2010	9	42	40	95	17	40	51	3.0
2011	8	40	39	98	17	43	45	2.6
2012	11	45	43	96	19	42	49	2.6
Overall		243	236	97	99	41	273	2.8

3 years=40.8%, 4 years=23.1%) with 4-year-olds significantly less likely to produce young compared to 2- or 3-year-olds (P<0.05). Between 2006 and 2012 only 12 litters were produced by 4-year-old females despite 52 breeding attempts of females of this age. Of these, all were produced by wild-born females but more wild 4-year-olds (n=45) were given breeding opportunities than captive-born females (n=7), due to the population age structure during this period. Of the 4-year-old females that produced litters, none produced a litter for the first time at this age. Most successful 4-year-old females (7/12; 58.3%) had produced litters at both 2 and 3 years, some had produced their first viable litter at 3 years (4/12; 33.3%) and one had produced a previous litter at 2 years only (1/12; 8.3%). Of unsuccessful 4-year-old females, one had produced a single litter at 1 year, 14 (35%) had produced a litter at 2 years only, five (12.5%) had produced a litter at 3 years only and four (10%) had produced litters at both 2 and 3 years. The remaining 40% (16 of 40) had no confirmed litters previously despite annual breeding opportunities since the age of 2 years. For females that produced PY at 3 years, most had successfully produced PY at 2 years as well (20/33; 60.6%), while some had produced litters for the first time at that age (8/33; 24.2%) after unsuccessful breeding opportunities at 2 years. The remaining females (5/33; 15.2%) were unlikely to have had breeding opportunities at 2 years as no previous breeding recommendations were in the records (therefore having their first breeding opportunity at 3 years). Of unsuccessful 3-year-old females, most (31/50; 62%) had not produced PY previously despite breeding opportunities.

Male effects on reproductive success

A total of 86 males were provided breeding opportunities with females during the study period, often with more than one female per year. Of the females that did not produce viable offspring, 51% were paired with males that had successfully produced offspring with at least one other female (proven sires), 23% were paired with males that never produced offspring (unproven sires) and 26% were paired with males of unknown fertility (e.g. records incomplete or non-specific). The average age difference between paired males and females was greater in successful pairings than unsuccessful pairings (males 0.5±0.1 versus 0.2±0.1 years older respectively; P=0.02). Males were confirmed to successfully produce young at ages 2 to 6 years. Of the 99 litters produced, 31 were produced by 2-year-old sires (31.3%), 33 were produced by 3-year-old sires (33.3%), 21 were produced by 4-year-old sires (21.2%), 9 were produced by 5-year-old sires (9.1%), 1 was produced by a 6-year-old sire and the remaining 4 litters had unknown paternity (due to pairing with two males).

Founder evaluation

The Tasmanian devil studbook lists a large number of devils that have never had the chance to contribute to the captive breeding programme. Some of these animals were brought into captivity for the purposes of breeding as part of the insurance population but died before having an opportunity to breed in captivity and therefore were removed as potential founders for analysis. Others were wild-caught females, likely cancer-positive, with PY, none of which survived to contribute to the captive population; these animals were not included in the analysis.

Of the 95 potential female founders, 26 (27.4%) never produced viable young. Of the 69 females which produced young, 18 (26.1%) of them no longer have viable founder lines as their offspring either died or failed to produce young of their own. As of 2012, only 51 (53.7%) female founder lines were still active from the original 95 female founders (Figure 2). Of the females that produced litters, most only produced a single litter (41 of 69; 59.4%). Although the cause of death is not listed in studbook records, wild females that were brought into captivity with PY and died within 6 months were likely to have been cancer-positive and died as a result of the cancer. A total of 14 female founders fit into this category and therefore only produced a single litter as a founder due to death preventing further contributions. A total of 19 (28%) females produced two litters, and 9 (13%) females produced three litters. The average litter size produced by a founder female during her reproductive life was 2.8±0.1 PY. Of the 298 PY produced by founder females (including wild-caught females with PY sired in the wild), there was no sex bias (P=0.4); 151 were male and 136 were female.

Of the 71 potential male founders, 53 (74.6%) produced offspring, 11 (15.5%) never produced viable PY and an additional 7 (9.9%) males not yet confirmed as sires were either young enough to have future breeding opportunities or were possibly sires of litters where the female had access to multiple males during the breeding season (Figure 2). Of the 53 founder males confirmed to have offspring, 3 (6%) had founder lines that were no longer viable. Therefore, as of 2012, 57 (80.3%) male founder lines were still active from the original 71 male founders. Males successfully produced PY from the ages of 2 to 6 years. Of the founder males that sired PY, 16 (30.2%) sired a single litter, 22 (41.5%) sired two litters, 12 (22.6%) sired three litters and 3 (5.7%) sired four litters.

Housing

Four out of nine surveys were returned during the study period, limiting information and the ability to conduct statistical analysis on the relationship between housing and breeding success.

Table 2. Number, age and average litter size of female Tasmanian devils recommended for breeding within the captive insurance programme in 2006-2012.

	Captive-born females	Wild-born females	2-year-old females	3-year-old females	4-year-old females
Total number with breeding recommendations	102	143	107	84	52
Total litters confirmed	39	59	51	34	12
Recommendations resulting in viable litters (%)	38.2	41.2	47.7	40.5	23.1
Average litter size	2.6±1.0	2.8±1.1	2.7±1.1	2.8±0.9	2.4±0.9

Regardless, there appears to be no obvious correlation between devil density and proximity, enclosure size and breeding success with all institutions demonstrating breeding success, although variable between years. For example, in 2009, Institution #1 held 12 female and 15 male adult devils, in addition to a number of juvenile devils and successfully bred 9 of the 12 females (75%; all 2-year-old females). Yet Institution #2 held three adult females in the same year (with individual males to pair with each) and only had a single female breed. Cases of successful females being housed in adjacent pens occurred in two of the four institutions. The only institution that had a consistent breeding rate was Institution #1, but only after breeding females were moved into new, purpose-built devil enclosures for the 2009 breeding season (breeding success prior to this was very low; 0–16% in 2007–2008). Since then (2009–2012), Institution #1 has consistently produced young from most females with breeding recommendations (72–89% success; 9 to 11 females paired per year) with 100% success rate from 2-year-old females (n=16), 72% success rate from 3-year-old females (n=18) and 57% success rate from 4-year-old females (n=7) during this time. The purpose-built enclosures (7×20 m or 140 m^2 each; built in a line on the 20 m shared fence line) in Institution #1 were used exclusively for breeding from 2009 onwards with female enclosures constructed with two den options, a concrete den with natural flooring and a dirt mound for building dens if desired.

The size of the pens which housed successful breeding female devils ranged from 24 m^2 (2008–2009 in one facility only) to 150 m^2. Female devils were provided with two den options, including either two man-made dens (e.g. concrete square den and clay pipe within dirt mound) or one man-made den and the materials provided so that the devil could construct its own den (e.g. concrete square den and large, compacted dirt mound). All man-made dens were made of either wood or concrete, with either a natural (dirt) floor or floor constructed of the same material as the rest of the den with a removable or hinged lid for management access. All enclosures had natural substrate (e.g. dirt) and logs, branches or bushes for climbing or shelter and shade. All enclosures had a fence made of smooth material to prevent escape (typically Colorbond® fencing) with a minimum height of 1.2 m. Overall, enclosure design (the amount of furniture, size and structure of dens) varied between facilities but all provided the minimum required by the recommended husbandry guidelines available at the time or by state regulations (Kelly 2007). Institution #3 had both a repurposed wombat breeding facility (2008 onward) and purpose-built (2010 onward) devil breeding facility (~15 m between the two) but only had breeding success in the original repurposed facility during the study period. The inclusions and size of the enclosures were similar with the exception of den design. Although enclosures in both facilities included a concrete pipe embedded into an earth mound as a den in each enclosure, the older facility had a tunnel complex with dens that had wooden walls and dirt floors and the newer facility had concrete dens with concrete floors as the secondary den (both facilities also had straw material in the den).

Discussion

There was low overall reproductive success in the first seven years (2006–2012) of the Tasmanian devil insurance programme with only 40.7% of breeding recommendations producing litters. Male founder lines persisted 30% more than female founder lines, however, the low breeding success and loss of founder lines is of concern in a species with limited genetic diversity (Jones et al. 2004). With little difference in breeding success and litter size between wild-caught versus captive-born females, it is unlikely that origin of birth affected breeding success in the programme's early stages. Females were more likely to successfully produce a litter if paired with an older male, suggesting male size or experience may be an important factor. Female age at first breeding appears more important to reproductive success and therefore the breeding of 2-year-old females should be prioritised in future breeding strategies.

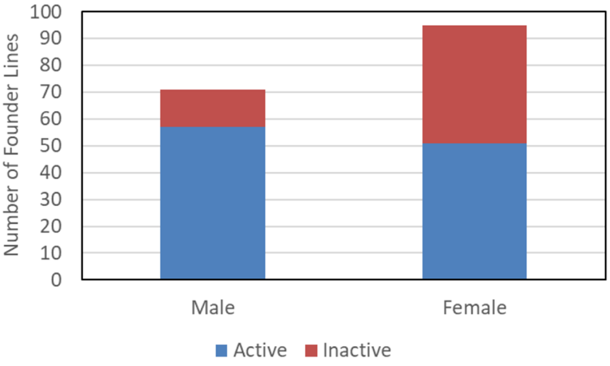

Figure 2. Total number of active and inactive founder lines for male and female devils at the end of the study period (2006–2012) demonstrating the loss of founder lines over time

Overall, the captive breeding programme is producing young at a lower rate and with smaller litter sizes than reported for wild devils (Keeley et al. 2017). A historic evaluation of litter production and size in wild devils before the transmissible cancer emerged reported a 75% success rate (females pouch checked and confirmed with PY) with an average of 3.4±0.9 PY per litter and a high proportion of litters with the maximum number of 4 PY (63%; Keeley et al. 2017). It is therefore possible that some artefact of captivity is preventing or limiting the successful production of young.

No difference was found between litter sizes of young born by captive-born or wild-caught females in 2006–2012. This contradicts a difference in litter size found in a recent study that examined the same population over a similar time frame (2006–2013) but also included devils held and bred in free-ranged and managed environmental enclosures (Hogg et al. 2015). They reported that 31.3% of breeding recommendations for wild-caught females successfully produced an average of 2.91±1.0 PY per litter and that 44.9% of recommendations for captive-born females successfully produced an average of 2.41±1.0 PY per litter (Hogg et al. 2015). It is possible that the differences between these two studies relate to inaccuracies in annual reports between 2006 and 2012 found in this study (corrected using studbook data) as well as the inclusion of additional animals from free-ranged and managed environmental enclosures in the other study. However, despite limited numbers of captive-born females in the programme during the first two years producing low numbers of PY per litter, in subsequent years their breeding success and PY per litter were similar to that of the wild-caught females, confirming that the shift from predominately wild-caught to captive-born females over time did not influence overall breeding success. The annual reports for 2011–2013 (Zoo and Aquarium Association) also reflect this trend; even with the inclusion of females in free-ranged and managed environmental enclosures, a large proportion of PY produced in these years were from captive-born females with an average PY per litter higher than that reported in the previous study (Hogg et al. 2015). So, contrary to the hypothesis that productivity was lower for captive-born females (Hogg et al. 2015) and that breeding success for captive-born females will decline over generations (Farquharson et al. 2017) the results of the current study suggest that productivity in devils, both chance of producing young and number of young per litter, between captive-born and wild-caught females is similar over time. This suggests that it should be possible to maintain a captive-born population without relying on bringing in new wild devils if that option becomes limited in the future. However, if the loss of founder lines continues at the current rate, closing the captive population may have a negative impact on its genetic diversity as founder alleles need to be retained within the captive population (Lacy 1989) by keeping founder lines active. Supplementing with new founders where possible aids in maintaining adequate genetic diversity as breeding only from captive-bred individuals can produce genetic adaptations to captivity in as little as three generations in intensively managed systems (Araki et al. 2007).

Interestingly, results show that female devils were more likely to be successful if paired with an older male rather than one of similar age. Although it was not feasible to evaluate body weight within this study, it is possible that this outcome is associated with greater body weight rather than age. Two-year-old males in captivity have often not yet reached full body mass (T. Keeley, personal observation) and therefore older males would tend to be larger. It has been reported in other dasyurid species that successful males are heavier than unsuccessful males—perhaps this is a female choice or sexual selection mechanism to improve future offspring survival (Glen et al. 2009; Kraaijeveld et al. 2003). Certain conditions prevented ruling out or examining other potential male-related factors: lack of a pregnancy test (Keeley et al. 2012a) and reliable semen collection technique (Keeley et al. 2012b) to examine male fertility in unsuccessful pairing attempts for which mating was observed; lack of older males (>5 years) with breeding recommendations; and lack of records of unsuccessful pairing attempts (e.g. only amicable pairing attempts or those for which mating or mating-related behaviours were observed were included in annual reports).

Devils in this captive breeding study were found to produce smaller litters and have reduced breeding success, regardless of dam birth origin, compared to wild devils (Keeley et al. 2017). As a pregnancy diagnostic tool has yet to be developed for this species, it is impossible to know if the lack of success in captivity is due to a lack of achieving pregnancy or a significant loss of PY at or shortly after birth. As pouch checks are not conducted for two weeks to two months after birth, it is impossible to quantify the number of litters that were born but lost prior to the initial pouch check. It is possible that new-born devils lost before the initial pouch check or between pouch checks may have been consumed by the mother (similar to the cannibalism observed early in the dibbler *Parantechinus apicalis* breeding programme; Lambert and Mills 2006) as new-born PY have never been found in the enclosures. In striped-face dunnart *Sminthopsis macroura* laboratory colonies, small litters (one to two PY) were never reared to weaning (Godfrey 1969; Woolley 1990), and 18 of 25 litters were lost or died at birth, possibly due to handling, stress or inbreeding (Godfrey 1969). Irregular cycles were common suggesting deleterious effects on reproduction associated with inbreeding (Godfrey 1969). Studies on the fat-tailed dunnart *Sminthopsis crassicaudata* showed similar results; pairings of close relatives (e.g. siblings, parent-offspring) were often successful but with reduced viability of offspring (Bennett et al. 1989). Similar factors could contribute to reduced reproductive success in devils. As the relatedness of founder devils was not confirmed by genetic analysis prior to breeding, it is possible that offspring had some level of inbreeding. If some founder devils were indeed siblings or half sibs (Hogg et al. 2015), this may have increased the rate of undetected inbreeding, leading to an increased number of unviable offspring in subsequent generations. A recent study of a wild, cancer-free population found a decline in reproductive success in recent years during which there was evidence of inbreeding depression within the population, specifically low microsatellite diversity with levels of internal relatedness constant between years (Farquharson et al. 2018; Gooley et al. 2020), supporting this hypothesis.

Within this study, most females produced young for the first time at 2 years, with no records of first breeding at 4 years . Records of 5-year-old female devils producing offspring are rare and limited to females that produced several litters in their lifetime, suggesting that female devil fertility decreases with age, affecting their reproductive history much like in cheetahs, rhinos and elephants (Hermes et al. 2004, Wachter et al. 2011). Asymmetrical reproductive aging has been observed in mega vertebrates, where in the wild most of the female's reproductive lifespan would be spent in pregnancy or lactation; decreasing the number of oestrous cycles experienced and potential deleterious effects of prolonged exposure to endogenous sex steroids during their lifetime (Hermes et al. 2004). In the lesser mouse lemur *Microcebus murinus*, artificial manipulation of seasonal reproduction using an accelerated photoperiodic regimen affected reproductive longevity, expressed as reproductive senescence at a younger age rather than through the number of seasonal cycles or reproductive potential (Perret 1997). If a species has a 'fixed' number of oestrous cycles in which reproductive potential is optimal, then extension beyond this due to prolonged periods of non-mating may accelerate reproductive and chronological aging. For captive devils, a female may experience the same number of oestrous cycles in captivity (three in a year) that she would experience in the wild if successfully breeding every year (three over three years, Keeley et al. 2012a; Lachish et al. 2009). This may have a negative effect on breeding success in subsequent years, reducing her ability to become pregnant or produce viable young as she ages.

Similar to the current findings in devils, breeding success in cheetahs has been related to reproductive history and the age at which females were first bred as opposed to a lack of genetic diversity, irregular hormone cycling or captivity-related stress (Crosier et al. 2011; Wachter et al. 2011). The occurrence of genital pathologies was more commonly observed in older and nulliparous females than in younger, reproductive animals demonstrating an effect of delayed reproduction on reproductive health as well as reproductive success in the cheetah (Crosier et al. 2011; Wachter et al. 2011). This demonstrates that first breeding at age of sexual maturity can be beneficial to both an animal's fecundity and future reproductive health (Crosier et al. 2011; Penfold et al. 2014; Wachter et al. 2011). Similar trends observed in devils confirm the need for early mating opportunities. A recent evaluation of breeding records in the Tasmanian devil had similar findings, although breeding success declined with age at first breeding but only in the evaluation of devils housed in intensive breeding institutions, not in the population as a whole (Farquharson et al. 2017). As that study only looked at a single breeding record for each female (age of first breeding), it is possible they would have confirmed this trend for the whole population if all records for all females were evaluated (Farquharson et al. 2017). Therefore to improve female reproductive success, it is advisable that female devils are provided with breeding opportunities at 2 years old. It is important that this consideration becomes incorporated into captive breeding recommendations and optimal breeding pair choices to maintain genetic diversity and captive breeding success in the long term. To be effective in the long term, this would mean that yearly breeding recommendations identify the best pairings at that time as well as projecting the impact of not breeding a female at 2 years (and therefore decreasing her potential to produce young in the future) on the genetic diversity of the population.

Although information regarding the captive environment and housing conditions were limited, it is noteworthy that increases in reproductive success at Institution #1 coincided with facility upgrades. For Institution #1, better suited dens (with dirt floors or ability to dig a den out of a dirt mound) and larger enclosures sizes (140 m^2) may have better replicated wild conditions. Devils will dig their own dens as well as occupy abandoned wombat burrows or caves, using the den during inactive periods and as a safe house for young (Pemberton 2019). It is not possible with the limited current data to rule out space use requirements (e.g. amount or nature of furniture) or proximity to other devils as other contributing factors; further exploration into enclosure design may be warranted.

With the complexity of enclosure size and design, transfer of animals between facilities for breeding, husbandry requirements, mate choice and limited genetic diversity, there are many interacting factors which may contribute to suboptimal breeding success in the Tasmanian devil. This study has demonstrated that although overall breeding rates are low, they are consistent. To ensure continuity, it is important that the reproductive biology of the species is incorporated into breeding recommendations. As the cancer is not vertically transmitted, additional founders are still available for incorporating into the insurance population. Unfortunately, this may not always be the case, therefore it is important to continue to investigate further factors which may influence reproductive success in this species.

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