Molecular Ecology (2007) **16**, 2998–3008 doi: 10.1111/j.1365-294X.2007.03365.x

Low genetic diversity in the bottlenecked population of endangered non-native banteng in northern Australia

COREY J. A. BRADSHAW,* YUJI ISAGI,†¶ SHINGO KANEKO,‡ BARRY W. BROOK,*,** DAVID M. J. S. BOWMAN*†† and RICHARD FRANKHAM§

*School for Environmental Research, Institute of Advanced Studies, Charles Darwin University, Darwin, NT 0909, Australia, †Graduate School of Integrated Arts and Sciences, Hiroshima University, Kagamiyama 1-7-1, Higashi-Hiroshima 739-8521 Japan, ‡Graduate School for International Development and Cooperation, Hiroshima University, Kagamiyama 1-5-1, Higashi-Hiroshima 739-8529, Japan, §Department of Biological Sciences, Macquarie University, NSW 2109, Australia

Abstract

Undomesticated (wild) banteng are endangered in their native habitats in Southeast Asia. A potential conservation resource for the species is a large, wild population in Garig Gunak Barlu National Park in northern Australia, descended from 20 individuals that were released from a failed British outpost in 1849. Because of the founding bottleneck, we determined the level of genetic diversity in four subpopulations in the national park using 12 microsatellite loci, and compared this to the genetic diversity of domesticated Asian Bali cattle, wild banteng and other cattle species. We also compared the loss of genetic diversity using plausible genetic data coupled to a stochastic Leslie matrix model constructed from existing demographic data. The 53 Australian banteng sampled had average microsatellite heterozygosity (H_E) of 28% compared to 67% for outbred *Bos taurus* and domesticated *Bos javanicus* populations. The Australian banteng inbreeding coefficient (*F*) of 0.58 is high compared to other endangered artiodactyl populations. The 95% confidence bounds for measured heterozygosity overlapped with those predicted from our stochastic Leslie matrix population model. Collectively, these results show that Australian banteng have suffered a loss of genetic diversity and are highly inbred because of the initial population bottleneck and subsequent small population sizes. We conclude that the Australian population is an important hedge against the complete loss of wild banteng, and it can augment threatened populations of banteng in their native range. This study indicates the genetic value of small populations of endangered artiodactyls established *ex situ*.

Keywords: augmentation, *Bos javanicus*, cattle, effective population size, endangered species, founder effect, genetic diversity, heterozygosity, inbreeding, Leslie matrix, projection model

Received 10 January 2007; revision received 6 March 2007; accepted 27 March 2007

Introduction

As populations of threatened species continue to decline due to human modifications to the biosphere (Ceballos & Ehrlich 2002; Thomas *et al*. 2004), biodiversity managers are increasingly turning to re-introduction strategies to conserve species in their native range (e.g. Lomolino & Channell 1998; Moritz 1999). However, recently established populations arising from (typically) a small number of founders are of concern because their long-term viability may depend on the initial magnitude and maintenance of genetic variation (Wright 1969; Nei *et al*. 1975; Frankel &

Correspondence: Corey J. A. Bradshaw, Fax: +61 88946 7720; E-mail: corey.bradshaw@cdu.edu.au

¶Present address: Laboratory of Forest Biology, Division of Forest and Biomaterials Science, Graduate School of Agriculture, Kyoto University, Kitashirakawa Oiwake-cho, Sakyo-ku, Kyoto 606-8502, Japan

**Present address: Research Institute of Climate Change and Sustainability, School of Earth and Environmental Sciences, University of Adelaide, SA 5005, Australia

††Present address: Department of Plant Science, University of Tasmania, Private Bag 05, Hobart, Tas. 7001, Australia

Soulé 1981). Populations derived from small numbers of founders typically have lower genetic variation because of genetic drift (Frankel & Soulé 1981; Williams *et al*. 2002; DeYoung *et al*. 2003; Wilson *et al*. 2005), and many existing populations of endangered species may already be genetically compromised. A recent meta-analysis of 170 pairs of threatened and related nonthreatened taxa revealed that average heterozygosity was 35% lower for threatened vs. nonthreatened species (Spielman *et al*. 2004). Increased homozygosity in small populations results in greater exposure of deleterious recessive alleles and reduced reproduction and survival (inbreeding depression) (Keller & Waller 2002). The effects of inbreeding depression on population viability are exacerbated by stressful environmental conditions (Armbruster & Reed 2005), and it is expected that the loss of genetic diversity will reduce species' capacity to adapt to future climate change (Rice & Emery 2003). However, not all genetically compromised populations show overt negative effects of inbreeding depression (Paetkau & Strobeck 1994; Berger & Cunningham 1995). Many species demonstrate low heterozygosity despite no concomitant evidence of population decline (Amos & Balmford 2001); but these observations do not exclude detrimental impacts because population growth only requires that the rate of change remains greater than zero. Thus, despite the consensus of the damaging genetic effects of small founder populations (Newman & Pilson 1997; Saccheri *et al*. 1998; Keller & Waller 2002; Frankham 2005; O'Grady *et al*. 2006), there remains uncertainty about its importance for the longer-term risk of extinction (Lande 1988; Caro & Laurenson 1994; Caughley 1994; Keller & Waller 2002).

The introduced banteng (*Bos javanicus*) population of northern Australia offers a rare opportunity to examine the relationship between inbreeding and population viability in a threatened species having been introduced to a region well outside of its native range. Approximately 20 banteng were brought to Australia from Bali, Indonesia in 1849 and subsequently released in northern Australia (Corbett 1995; Fig. 1). Recently, we determined that Australian banteng were genetically consistent with wild banteng in Southeast Asia and that they demonstrated no evidence of hybridization with other *Bos* spp. (Bradshaw *et al*. 2006). We argued that this places the Australian population in a unique context that demands careful conservation assessment because it is a non-native species thriving *ex situ*, while its wild, parent population is currently listed as Endangered (IUCN 2005). In Southeast Asia today, there are estimated to be < 5000 pure-strain *B. javanicus* in the wild scattered among small (< 500 individuals), disjunct populations (Hedges 1996; IUCN 2005), so the Australian population of *c*. 6000 individuals (adjusted from that reported in Bradshaw & Brook 2007) represents the largest extant population in the world (Bradshaw *et al*. 2006).

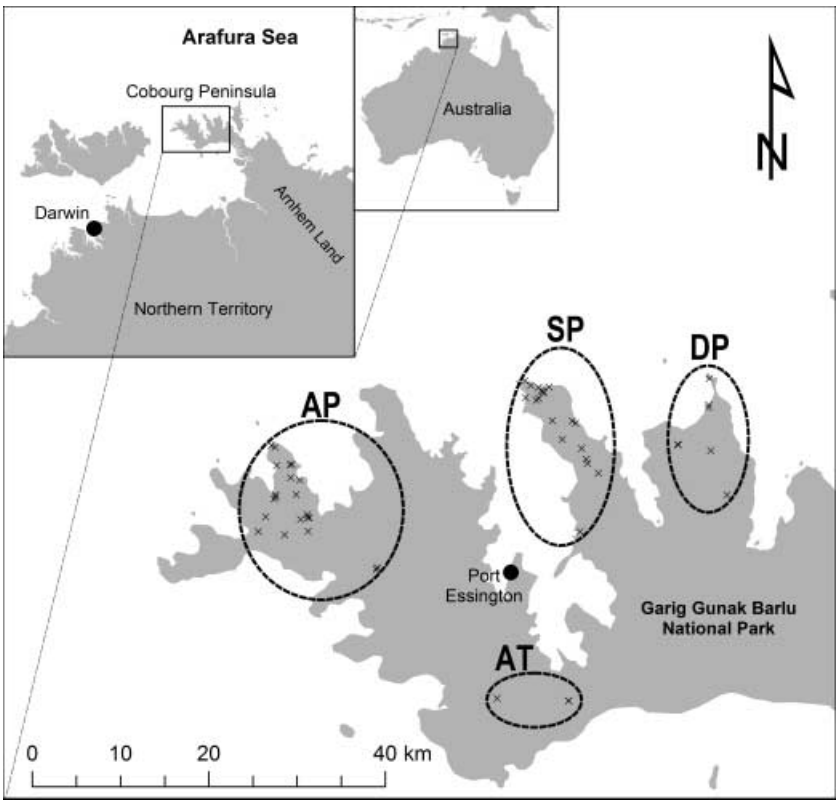

Fig. 1 Map of Garig Gunak Barlu National Park on the Cobourg Peninsula, Northern Territory, Australia showing the locations of tissue samples (black crosses) collected from free-ranging banteng (*Bos javanicus*). The four main clusters of samples were grouped as Araru Point (AP, $n = 23$), Araru Turn-off (AT, $n = 3$), Smith Point (SP, $n = 21$) and Danger Point (DP, $n = 6$).

To determine the genetic value of the Australian banteng as a conservation resource, we (i) predicted the expected loss of genetic diversity from the founding Australian banteng population using a Leslie matrix population model and empirical measurements of expected initial heterozygosity; (ii) determined whether the population's measured levels of microsatellite genetic diversity fell within the expected range; and (iii) examined how these measured levels compared with published genetic information from other artiodactyls. Our a priori expectation is that the bottlenecked Australian banteng population should exhibit low levels of heterozygosity, but enough to allow their subsequent population increase and expansion over a period of more than 150 years (Bradshaw & Brook 2007). Further, we discuss the potential for management-regulated genetic exchange between wild Southeast Asian and Australian populations.

Materials and methods

Population history

Shortly after introduction of livestock to Port Essington (Victoria Settlement) on the Cobourg Peninsula of the Northern Territory, Australia (Fig. 1), the small settlement was abandoned in 1849 and all cattle, Timor ponies (*Equus caballus*), pigs (*Sus scrofus*), poultry (*Gallus* spp.), swamp buffalo (*Bubalus bubalis*) and banteng were left behind (Kirby 1979). The *Bos javanicus* population was largely forgotten by European Australians until they were rediscovered to science in 1960 (Letts 1964). During the interval between introduction and rediscovery, the population increased to at least 3000 animals, despite hunting by resident indigenous people. Yet, they also remained in virtual isolation on the Cobourg Peninsula, failing to spread to other parts of the Northern Territory (Kirby 1979). Recent work confirms that the population has been genetically isolated from congeners, with no evidence of cross-species introgression (Bradshaw *et al*. 2006). The population is now estimated to number between 5000 and 7000 individuals, the numbers having been adjusted downward after determining an over-estimated sightability bias in the population size reported in Bradshaw & Brook (2007) (K. Saalfeld, unpublished data).

Data collection

We collected 53 skin samples from free-ranging banteng (41 male, 12 female) in Garig Gunak Barlu National Park (~220 000 ha), Cobourg Peninsula, Northern Territory, Australia (11°20′S, 132°20′E, Fig. 1) using remote biopsy darts (Bradshaw *et al*. 2006), or after direct capture via chemical immobilization and manual biopsy (Bradshaw *et al*. 2005). Samples were collected from four distinct areas within the park (Fig. 1): Araru Point (AP), Araru Turn-off (AT), Smith Point (SP) and Danger Point (DP), locations separated by obvious geographical boundaries (e.g. major inlets, large open areas largely devoid of water in the dry season). Samples were stored in 90% ethanol prior to DNA extraction.

DNA extraction and microsatellite genotyping

DNA was extracted and purified using standard SDS/proteinase K protocol and phenol–chloroform extractions (Sambrook & Russell 2001). Twelve polymorphic microsatellite loci that were originally isolated from N'Dama taurine cattle (ILSTS001, ILSTS005, ILSTS006, ILSTS011, ILSTS019, ILSTS022, ILSTS033, ILSTS049, ILSTS058, ILSTS078, ILSTS087, ILSTS103; Brezinsky *et al*. 1993; Kemp *et al*. 1995) were genotyped (three of which, ILST005, ILST006 and ILST011, have also been shown to be polymorphic across European cattle species; Maudet *et al*. 2004; Tapio *et al*. 2006). Polymerase chain reaction (PCR) amplifications were performed under the following conditions: initial denaturation at 95 °C for 9 min; then 30 cycles of denaturation at 92 °C for 30 s, annealing at primer-specific temperature for 30 s (58, 55, 55, 58, 50, 58, 55, 55, 55, 55, 58, and 55 °C for each primer pair listed above, respectively), extension at 72 °C for 1 min, and final extension at 72 °C for 8 min. The size of PCR products was measured using a 3100 Genetic Analyser with GENESCAN analysis software (Applied Biosystems).

Reference data

To compare the genetic diversity of Australian banteng to an outbred population of approximately the same expected heterozygosity as the true source population (for which no data exist), we obtained data from 15 polymorphic microsatellite loci genotyped from 17 domesticated Bali cattle from Malaysia and 4 wild-originated banteng housed in the Blijdorp Zoo in Rotterdam, the Netherlands (data courtesy of J. A. Lenstra, I. J. Nijman and O. Hanotte; see Nijman *et al*. 2003). The loci examined were ILSTS005, ILSTS006, ILSTS008, ILSTS033, ILSTS023, ILSTS028, ILSTS036, ILSTS050, ILSTS103, AGLA293, MGTG4b, TGLA48, TGLA122, TGLA126, and TGLA227. From these data we calculated observed (H_O) and expected heterozygosities (H_E), and the number of alleles (A) for each locus and averaged over all loci (see Results). However, the Bali cattle samples were derived from *Bos javanicus-indicus* hybrids (Nijman *et al*. 2003), and the study used different samples of loci, which makes these data an imperfect reference source to compare to Australian banteng. We also compared genetic diversity in the Australian banteng with those for outbred populations of *Bos taurus* (see below).

Predicted loss of genetic diversity

To derive a predicted loss of genetic diversity based on the known size of the founding population and its subsequent rate of increase, we used a pre-existing stochastic Leslie matrix (age-structured) population model (Caswell 1989) constructed for Australian banteng (Bradshaw & Brook 2007). The full Leslie matrix incorporated both sexes with longevity capped at 17 years, and assumed an environmental carrying capacity (K) of 6000 individuals. The model included negative density-dependent feedback in survival and fertility, demographic stochasticity in survival and fertility, stochastic variation in survival with rainfall, and an episodic catastrophic mortality frequency forecasted from a pre-established catastrophe-generation time relationship (Bradshaw & Brook 2007). The model predicts a relatively rapid rate of increase with the population achieving carrying capacity 50–60 years after introduction, and a generation time (i.e. mean age of the parents of the offspring produced by the population at the stable age distribution) of 7 years (Bradshaw & Brook 2007).

To evaluate the potential degradation in heterozygosity, we used the Malaysian Bali cattle reference data (Nijman *et al*. 2003) combined with heterozygosity values obtained from the literature to provide a reasonable H_E for comparable outbred conspecific and congeneric populations. A recent genetic investigation of 11 'safe' breeds (those not considered to be at risk of extinction) of northern European cattle (*B. taurus*) showed an average microsatellite heterozygosity of 0.67 (± 0.04 SD; see Results) (European Cattle Genetic Diversity Consortium 2006; Tapio *et al*. 2006). The Bali cattle reference data provided a nearly identical value of $H_E = 0.68$. Assuming these values are representative of genetically healthy populations of genus *Bos*, we used the value of 0.67 as initial heterozygosity (H_0). To estimate the expected proportionate reduction in genetic diversity with each passing generation, we estimated the effective population size (N_e) from the number of adult females and males. The adult sex ratio modifies N_e as:

$$N_e = \frac{4N_m N_f}{N_m + N_f} \qquad \text{(eqn 1)}$$

where N_m = the number of reproductive males and N_f = the number of reproductive females (Wright 1969). However, the normal tertiary sex ratio $[N_f/(N_f + N_m)]$ for large polygynous ungulates is 0.83 (Bessa-Gomes *et al*. 2004), which is due to the polygynous breeding system that arises when more competitive males monopolize female harems, thus reducing the number of males contributing to subsequent generations. Therefore, the founding N_e (assuming equal numbers of adult female and males were brought to Australia at introduction) is 7 (6.8). The proportionate reduction in H_E after one generation becomes $1/(2N_e)$ (Wright 1931); for example, a reduction of 0.07 in H_0 is expected after one generation with a founding $N_e = 7$, leading to $H_1 = H_0 \times (1 - 0.07) = 0.62$ (assuming $H_0 = 0.67$).

N_e is also a function of fluctuations in N_e over generations, so we calculated overall N_e as the harmonic mean of those values estimated for each generation. Another important factor affecting N_e is variation in family size (i.e. lifetime production of offspring per individual; Frankham *et al*. 2002). Variation in family size has been shown to reduce effective population size by an average of 54% over a range of species (Frankham 1995). Given the lack of specific data with respect to this phenomenon in *Bos* spp., we further adjusted N_e such that $N'_{e,t} = 0.46 \cdot N_{e,t}$ (Frankham 1995). The stochastic simulation estimating the range in expected N_e was based on 1000 iterations of the model. While age structure and the overlapping generations that result can potentially influence effective population size, Frankham (1995) failed to find evidence for an effect of overlapping generations on the N_e:N ratio. Although there are several methods to deal with this potential problem (Caballero 1994; Engen *et al*. 2005), we did not have detailed information on age structure and overlapping generations, so we ignored these factors.

Sensitivity analysis

To examine the sensitivity of N_e predictions to the starting parameters used, we performed sensitivity analyses where we modified (i) the initial sex ratio of the founding population of 20 individuals (sex unknown) to favour females over males by a factor of approximately 2 (i.e. 14 females, 6 males) — this could be expected if the colonists had favoured females for breeding stock over males; and (ii) the initial expected heterozygosity by ±10% of 0.67 (i.e. 0.60–0.74), which effectively encompasses the range expected for outbred cattle populations (European Cattle Genetic Diversity Consortium 2006).

Genetic data analysis

The number of alleles per locus (A), observed heterozygosity (H_O) and expected heterozygosity, were calculated to quantify the genetic variation within populations. Heterozygosity measures were corrected for sample size as recommended by Nei (1978). Deviation from Hardy–Weinberg expectations and linkage equilibrium between loci (random associations of alleles at different loci in gametes) were tested with FSTAT (version 2.9.3; Goudet 1992). To compare the allelic diversity (A) of Australian banteng directly to the sample of 17 Malaysian Bali cattle, we performed a rarefaction analysis on the Australian banteng samples to correct for unequal sample sizes (individuals) between them (Leberg 2002). We randomly sampled (without replacement) 17 individual Australian banteng from our sample of 53 and calculated

the allelic diversity (A) at each locus sampled, and then calculated the average A over all loci. This process was repeated 10 000 times. Analysis of molecular variance (AMOVA; Excoffier *et al*. 1992) was used to examine the hierarchical genetic structure with program GENALEX version 6.0 (Peakall & Smouse 2006). Genetic variation was partitioned into two levels: among and within populations. To analyse the genetic differentiation among populations (both four-way and two-way comparisons; see Results), overall and pairwise F_{ST} values were also calculated, and the probability that each pairwise F_{ST} value was not greater than zero was calculated using permutation tests (Peakall & Smouse 2006). F_{ST} probabilities were adjusted using Bonferroni correction for multiple testing.

Results

Predicted loss of genetic diversity

After 22 generations (7 years/generation), the stochastic Leslie matrix population model predicted H_E to range from 0.27 to 0.34 (mean = 0.32), with an increasing width of the 95% confidence intervals over time because of the cumulative effects of stochastic fluctuations in population size (Fig. 2). The overall effective population size (N_e) over the 22 generations modelled (harmonic mean corrected for variation in family size) ranged from 10.8 to 14.8 (mean = 13.3).

The sensitivity analysis modifying the initial sex ratio of the founding population to 14 females and 6 males shifted the predicted mean H_E upwards by 16% to 0.37 (95% confidence interval: 0.31–0.40), corresponding to an $\hat{N}_e$ of 13–19. Modifying H_0 itself by ±10% (H_0 ranging from 0.60 to 0.74) modified the predicted heterozygosity after 22 generations (H_{22}) by ±9–13% (when $H_0 = 0.60$, $\hat{H}_{22} = 0.24$–0.31; when $H_0 = 0.74$, $\hat{H}_{22} = 0.29$–0.38), but overall $\hat{N}_e$ after 22 generations was relatively invariant to these changes ($\hat{N}_e = 13.3$).

Microsatellite genetic variation

As predicted, the overall microsatellite genetic diversity of *Bos javanicus* populations in northern Australia was extremely low, having one to three alleles per locus, with an average of 1.83 (Table 1). The rarefied A to a sample size of 17 (for comparison to Malaysian Bali cattle; Table 1) ranged from 1.67 to 1.83. The observed and expected heterozygosities (H_O and H_E) at the loci examined ranged from 0.00 to 0.53 and from 0.00 to 0.50, with averages of 0.24 and 0.28, respectively (Table 1). The discrepancies between some H_O and H_E were checked for typing errors — none were found; therefore, the disparity may be due to small sample size in two of the subpopulations, and possible Wahlund effects. These measures of genetic diversity are much lower than those expected from an outbred population of Malaysian Bali cattle ($A = 5.27$,

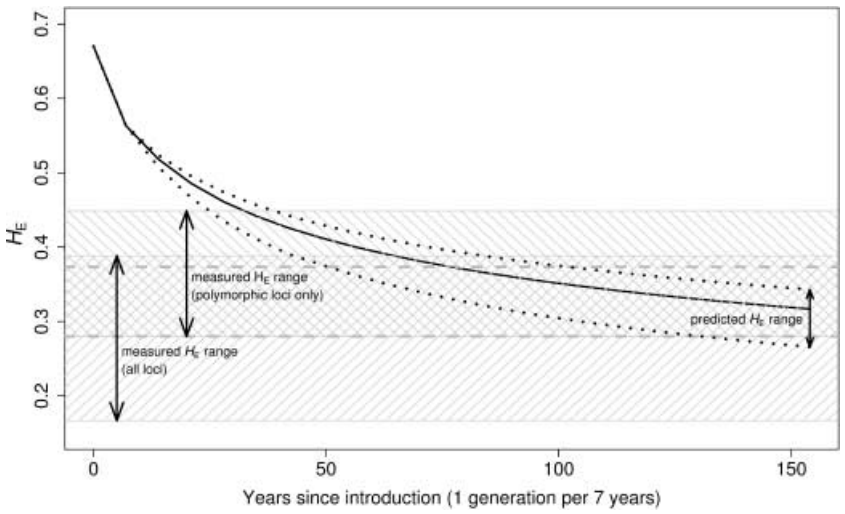

Fig. 2 Predicted reduction in expected heterozygosity (H_E) after 22 generations (H_{22}) (7 years per generation) assuming a starting H_E (H_0) = 0.67 (Table 2 and Tapio *et al*. 2006). A stochastic Leslie matrix model projected the founding population ($N = 20$) forward to the present, with effective population size (N_e) and the corresponding proportionate reduction in genetic diversity calculated after each generation (1000 iterations). This gave a mean (solid black line) and 95% confidence interval for the predicted H_E after each generation. Predicted H_E after 22 generations ($\hat{H}_{22}$) = 0.32 ranged from 0.27 to 0.34 (95% confidence limits). Also shown are the mean measured values of H_E (horizontal dashed grey lines) derived from 12 microsatellite loci ('all loci', $\bar{H}_E = 0.28$) and 9 polymorphic loci ('polymorphic loci only', $\bar{H}_E = 0.37$). Confidence intervals (95%) for the mean measured H_E are estimated using 10 000 bootstrap (with replacement) iterations (hatched polygons).

Table 1 Genetic variation for 12 microsatellite loci for *Bos javanicus* in northern Australia, in each of four regions of the Cobourg Peninsula (Fig. 1). Shown are the loci ID, number of individuals genotyped (n) for each locus, number of alleles per locus (A), observed heterozygosity (H_O) and expected heterozygosity at Hardy–Weinberg equilibrium (H_E). A ($P < 0.05$) deviation from Hardy–Weinberg equilibrium expectations is indicated by an asterisk

	AP				AT				SP				DP				Total			
Locus	n	A	H_O	H_E	n	A	H_O	H_E	n	A	H_O	H_E	n	A	H_O	H_E	n	A	H_O	H_E
ILSTS001	23	3	0.57	0.57	3	2	0.33	0.33	21	2	0.19	0.18	6	3	0.50	0.55	53	3	0.40	0.44
ILSTS005	23	2	0.35	0.35	3	2	0.67	0.53	21	2	0.38	0.46	6	2	0.33	0.48	53	2	0.38	0.43
ILSTS006	23	2	0.26	0.23	3	2	0.33	0.33	21	2	0.57	0.50	6	2	0.17	0.41	53	2	0.38	0.39
ILSTS011	23	1	0.00	0.00	3	1	0.00	0.00	21	1	0.00	0.00	6	1	0.00	0.00	53	1	0.00	0.00
ILSTS019	23	2	0.35	0.46	3	2	0.33	0.33	21	2	0.29	0.25	6	2	0.33	0.30	53	2	0.32	0.36
ILSTS022	23	2	0.04	0.04	3	2	0.67	0.53	21	2	0.19	0.25	6	2	0.17	0.53	53	2	0.15	0.23
ILSTS033	23	2	0.57	0.51	3	2	0.33	0.60	21	2	0.43	0.51	6	2	0.83	0.53	53	2	0.53	0.50
ILSTS049	23	2	0.22	0.37	3	2	0.00	0.53	21	2	0.43	0.51	6	1	0.00	0.00	53	2	0.26	0.44*
ILSTS058	23	1	0.00	0.00	3	1	0.00	0.00	21	1	0.00	0.00	6	1	0.00	0.00	53	1	0.00	0.00
ILSTS078	23	2	0.09	0.16	3	1	0.00	0.00	21	1	0.00	0.00	6	1	0.00	0.00	53	2	0.04	0.07
ILSTS087	23	2	0.35	0.51	3	2	0.67	0.53	21	2	0.43	0.51	6	2	0.50	0.53	53	2	0.42	0.50
ILSTS103	23	1	0.00	0.00	3	1	0.00	0.00	21	1	0.00	0.00	6	1	0.00	0.00	53	1	0.00	0.00
Average		1.83	0.23	0.27		1.67	0.28	0.31		1.67	0.24	0.26		1.67	0.24	0.28		1.83	0.24	0.28
SD		0.58	0.21	0.22		0.49	0.28	0.25		0.49	0.21	0.22		0.65	0.27	0.25		0.58	0.19	0.21

AP, Araru Point; AT, Araru Point Turn-off; SP, Smith Point; and DP, Danger Point (Fig. 1).

$H_O = 0.67$, $H_E = 0.68$; Table 2), and even lower than that of putatively inbred and highly endangered wild banteng ($A = 2.42$, $H_O = 0.48$, $H_E = 0.47$; Table 2). All Australian banteng microsatellite loci conformed to Hardy–Weinberg expectations within populations, and linkage disequilibrium among loci was not detected (Table 1); although we acknowledge that our power to detect linkage disequilibrium using a small number of random markers may have been low (Balloux *et al*. 2004). Even after excluding the monomorphic loci (ILST011, ILST058 and ILST103), average allelic diversity, H_O and H_E were still low (2.11, 0.32 and 0.37, respectively). The bootstrapped (10 000 iterations resampled with replacement) confidence intervals of estimated heterozygosity (calculated from all loci and polymorphic loci only) overlapped the predicted range in H_E derived from the Leslie matrix model (Fig. 2).

As a validation of the predictions of the stochastic Leslie matrix model, we also estimated overall N_e according to the relationship (Borlase *et al*. 1993):

$$\frac{H_t}{H_0} = e^{-t/2N_e}. \qquad \text{(eqn 2)}$$

Using $t = 22$ generations, initial expected heterozygosity (H_0) = 0.67 (combined result from Tables 2 and 3), and the measured H_t after 22 generations = 0.28, N_e becomes 12.6. This also results in an inbreeding coefficient (F) of 1 – 0.28/0.67 = 0.58. This result agrees well with the N_e range of 10.8–14.8 predicted by the model. Taking the rounded value of $N_e = 13$ and the harmonic mean of the overall population = 371 (harmonic mean number of individuals in the entire population over time from introduction to the present as estimated from the Leslie matrix projection), the ratio N_e:N is estimated to be 0.035.

Table 2 Genetic variation for 15 microsatellite loci for Malaysian Bali cattle and four wild-originated captive banteng from Blijdorp Zoo (Rotterdam, the Netherlands) (data from Nijman *et al*. 2003) showing locus ID, number of individuals genotyped (n) for each locus, number of alleles per locus (A), observed heterozygosity (H_O) and expected heterozygosity at Hardy–Weinberg equilibrium (H_E)

	Malaysian Bali cattle				Wild banteng			
Locus	n	A	H_O	H_E	n	A	H_O	H_E
ILSTS005	16	5	0.81	0.76	4	3	0.25	0.61
ILSTS006	17	6	0.76	0.63	3	3	1.00	0.73
ILSTS008	17	5	0.71	0.66	3	1	0.00	0.00
ILSTS033	16	2	0.50	0.39	4	2	0.50	0.43
ILSTS023	17	5	0.59	0.79	3	3	0.67	0.60
ILSTS028	17	5	0.76	0.68	3	3	0.67	0.80
ILSTS036	17	5	0.88	0.74	3	4	0.67	0.80
ILSTS050	17	8	0.53	0.79	3	3	1.00	0.73
ILSTS103	17	4	0.24	0.32	—	—	—	—
AGLA293	15	7	0.93	0.86	—	—	—	—
MGTG4b	16	6	0.69	0.74	3	1	0.00	0.00
TGLA48	17	5	0.59	0.74	—	—	—	—
TGLA122	17	6	0.76	0.78	3	3	0.67	0.60
TGLA126	17	7	0.71	0.71	3	2	0.33	0.33
TGLA227	17	3	0.65	0.63	3	1	0.00	0.00
Average		5.27	0.67	0.68		2.42	0.48	0.47
SD		1.53	0.17	0.15		1.00	0.36	0.31

Table 3 Measures of genetic variation in depleted or in re-introduced ruminant (Order Artiodactyla, Suborder Ruminantia, Families Bovidae and Cervidae) species, relative to their source or comparison populations. Shown are n, founding population size or N_e = depleted effective population size (in brackets), s, sample size (individuals sampled), Bottlenecked, introduced or depleted population, Non-bottlenecked, source or comparison population, H_E, expected heterozygosity, A, allele frequency

Family / Subfamily	Species	n or (N_e)	s	Bottlenecked H_E	Non-bottlenecked H_E	Bottlenecked A	Non-bottlenecked A	Source
Bovidae								
Bovinae	*Bos javanicus*	20	53	0.28	0.67	1.8	—	This study
	Bos taurus	(8)	13	0.01	0.70	—	—	Visscher *et al*. 2001
	Bubalus bubalis	< 80	23	0.43	0.54	3.0	4.2	Barker et al. 1997a
	Bison b. athabascae	37	30	0.52	0.55	3.6	6.6	Wilson & Strobeck 1999
	Bison b. athabascae	16	28	0.44	0.55	4.3	6.6	Wilson & Strobeck 1999
	*Syncerus caffer**	(20)	19	0.69	0.78	6.7	7.2	Van Hooft *et al*. 2000
	Syncerus caffer†	(75)	38	0.55	0.78	4.4	7.2	O'Ryan *et al*. 1998
	Syncerus caffer†	(23)	23	0.45	0.78	3.1	7.2	O'Ryan *et al*. 1998
Antilopinae	*Gazella dorcas*‡		8	0.53	—	3.8	—	Beja-Pereira *et al*. 2004
Caprinae	*Ammotragus lervia*‡		8	0.63	—	4.7	—	Beja-Pereira *et al*. 2004
	Ovis canadensis	12	20	0.43	0.59	2.1	4.6	Forbes *et al*. 1995
Average ± SE				0.45 ± 0.18	0.64 ± 0.09	3.8 ± 0.4	5.7 ± 0.9	
Cervidae								
Cervinae	*Cervus elaphus*	(15)	28	0.53	0.85	3.3	5.5	Hmwe *et al*. 2006
	Cervus e. nelsoni	34	55	0.25	0.56	1.9	3.4	Williams *et al*. 2002
	C. Nippon§	(90)	21	0.19	0.60	1.8	5.1	Goodman *et al*. 2001
	Rangifer t. tarandus¶	263	30	0.47	0.43	3.6	2.8	Jepsen *et al*. 2002
Odocoileinae	*Alces alces*	18	39	0.35	0.40	—	—	Broders *et al*. 1999
Average ± SE				0.36 ± 0.14	0.57 ± 0.18	2.7 ± 0.9	4.2 ± 1.3	

*Average values of north and south Kruger National Park (Van Hooft *et al*. 2000); †comparison population values from Van Hooft *et al*. (2000); ‡populations reduced in size to achieve threatened status; N_e unknown; §comparison of *C. n. nippon* from Nagasaki (Kyushu, Japan; $\hat{N}_e$ = 90) to *C. n. centralis* of Hyogo (Honshu, Japan; $\hat{N}_e$ = 970); ¶comparison of introduced *R. t. tarandus* (averaged over both north and south populations) to isolated and reduced populations of native *R. t. groenlandicus* (Jepsen *et al*. 2002).

Genetic structure

At the population level, the mean values of heterozygosity (H_O and H_E) over all loci were similar in all populations, whereas H_O and H_E of each locus were rather different among populations (for example, expected heterozygosities at ILSTS022 were 0.04 in AP, 0.53 in AT, 0.25 in SP, and 0.53 in DP). When examining all four subpopulations together, most of the observed variability occurred within populations (93.0% of the total AMOVA variance; Table 4). The result was similar when the analysis was restricted to the two larger-sampled subpopulations (AP and SP; Table 4). The overall F_{ST} estimate for the four-way comparison was 0.066 (P = 0.002), indicating restricted gene flow between populations, while the comparison involving the two larger-sampled subpopulations yielded F_{ST} = 0.090 (P < 0.001; Table 4). The population pairwise F_{ST} values ranged from 0.000 to 0.088, and two pairs of the six pairwise F_{ST} were larger than zero at the Bonferroni-corrected P < 0.05, and an extra pairwise difference at Bonferroni-corrected P < 0.10 (Table 5). Overall, there is strong evidence for genetic differentiation in the population (Fig. 1).

Discussion

As expected from a founding population of only 20 individuals, the average genetic variation of the Australian banteng population is particularly low compared to other populations of this and related species (average expected heterozygosity of only 0.28; Table 1). The expected microsatellite heterozygosity in small founding or depleted populations of artiodactyls (Order: Artiodactyla) within the same (Bovidae), and closely related (Cervidae) families (a taxon that shows a high proportion of primers amplifying microsatellite loci among species; Slate *et al*. 1998) was ~0.45 for bovids (including our results) and ~0.36 for cervids (Table 3). By comparison, larger related populations had average H_E of ~0.64 (bovids) and ~0.57 (cervids) (i.e. 37–42% higher; Table 3). The results for the four wild-originated, pure-strain *Bos javanicus* individuals show lower genetic diversity than their domesticated counterparts (Bali cattle), possibly because of the introgression of *Bos indicus* in the latter, but overall diversity was still much higher than that found in Australian banteng. This reduction in genetic diversity of the pure-strain

Table 4 Distribution of genetic diversity within and among (a) the four banteng subpopulations (Araru Point, Araru Point Turn-off, Smith Point and Danger Point; see Fig. 1) and (b) between the best-sampled populations of Araru Point and Smith Point, as determined by analysis of molecular variance (AMOVA). The total genetic diversity was partitioned among populations and among individuals within populations. Shown are the degrees of freedom (d.f.), sum of squares (SS), mean sum of squares (MS), genetic variability among populations (Var), percentage variability among populations, fixation index (F_{ST}), and test probability (P) for both comparisons

Source of variance	d.f.	MS	Var	Percentage
(a) Four subpopulations				
Among populations	3	4.204	0.115	7
Within populations	102	1.609	1.609	93
			$F_{ST} = 0.066$	
			$P = 0.002$	
(b) AP vs. SP only				
Among populations	1	8.295	0.157	9
Within populations	84	1.571	1.571	91
			$F_{ST} = 0.090$	
			$P < 0.001$	

Table 5 Pairwise F_{ST} (fixation index) values (lower diagonal) and P values (upper diagonal, in italics) among regional populations of *Bos javanicus* at Cobourg Peninsula, northern Australia. AP, Araru Point; AT, Araru Point Turn-off; SP, Smith Point; and DP, Danger Point (Fig. 1)

Region	AP	AT	SP	DP
AP	—	*0.152*	*0.001*	*0.072*
AT	0.051	—	*0.343*	*0.432*
SP	0.088	0.015	—	*0.016*
DP	0.045	0.000	0.080	—

B. javanicus is expected given the severe reductions in population size and range of populations in Southeast Asia that have likely suffered multiple bottlenecks themselves in the recent past.

Thus, the Australian population of *B. javanicus* is one of the more inbred populations of large, wild bovid examined to date (predicted inbreeding coefficient = 0.58). The only other semiwild bovid to display lower genetic diversity is the feral herd of Chillingham cattle (*Bos taurus*) in the north of England ($H_E = 0.013$; Visscher *et al*. 2001) because of a 300-year genetic isolation of this population of 49 individuals and a genetic bottleneck suffered during a crash to 13 individuals (Visscher *et al*. 2001). Other well-sampled species among the Bovidae and Cervidae with similar levels of inbreeding to Australian banteng were two introduced and bottlenecked populations of cervid, *Cervus elaphus nelsoni* and *Alces alces* of eastern North America (Broders *et al*. 1999; Williams *et al*. 2002). However, not all species introduced in small numbers to new or historical areas necessarily demonstrate such pronounced genetic erosion. Indeed, introduced populations of swamp buffalo in Australia (Barker *et al*. 1997a; Barker *et al*. 1997b), wood bison in Canada (*Bison bison athabascae*, Wilson & Strobeck 1999; Wilson *et al*. 2005), red deer in Italy (*Cervus elaphus*, Hmwe *et al*. 2006) and Bennett's wallabies in New Zealand (*Macropus rufogriseus rufogriseus*, Le Page *et al*. 2000) all had much greater genetic diversity than Australian banteng (Table 3).

Low genetic variability is usually found in extremely range-restricted or highly endangered populations (e.g. Ciofi & Bruford 1999; Eldridge *et al*. 1999), or in populations affected by severe fluctuations resulting in low effective population sizes (e.g. Holm *et al*. 1999). The low genetic variation in Australian banteng most probably results from genetic drift because we found no evidence for non-random mating within subpopulations, and polygynous ungulates are not expected to demonstrate nonrandom mating with respect to genotype (e.g. Paterson & Pemberton 1997). Admittedly, the ability to detect nonrandom mating depends to some degree on the level of heterozygosity present, yet this assumption has been upheld in other studies examining populations with similar levels of genetic diversity to Australian banteng (e.g. hairy-nosed wombat, *Lasiorhinus krefftii*, Taylor *et al*. 1994; Taylor *et al*. 1997). Despite reduced genetic variation, it is clear that the banteng population has thrived since its introduction to Australia over 150 years ago (Bradshaw *et al*. 2006; Bradshaw & Brook 2007). There appear to be no overt signs of fitness reduction related to the low heterozygosity observed, although subtle reductions in survival and fecundity due to inbreeding are plausible. If inbreeding depression had caused a reduction in reproductive fitness in Australian banteng, but not to the degree that the population's intrinsic rate of population increase fell below replacement levels, then its impact would be difficult to measure without reference to the recovery dynamics of a similar, but outbred population.

We observed clear subpopulation structure in the Australian population of banteng even within a relatively restricted area (2200 km^2), and this is likely to have resulted from high breeding-site fidelity, philopatry, and restricted dispersal capacity. Indeed, field observations of foraging behaviour demonstrate a highly restricted daily movement pattern (C. J. A. Bradshaw & D. M. J. S. Bowman, unpublished). Furthermore, the banteng population has never spread beyond the confines of the Cobourg Peninsula, in stark contrast to other introduced ungulates such as swamp buffalo and pigs that have spread across much of northern Australia. This apparently low capacity for broad-scale movements likely exacerbates the inbreeding potential of banteng relative to other ungulates.

We were able to predict the measured heterozygosity of the Australian banteng population by coupling published genetic data for other *Bos* spp. with a demographic model (Fig. 2). The extent of the predicted reduction in genetic diversity is still likely upwardly biased for two reasons: (i) Although we have good reason to believe that H_0 was around 0.67, it is still possible that the founding individuals were derived from a population that had itself gone through past bottlenecks. Predicted H_E was sensitive to variation in H_0 by approximately an equivalent amount, although overall predicted N_e was relatively insensitive to H_0; (ii) There is anecdotal evidence of periodic die-offs and large culls during the latter portion of the banteng's history at Cobourg Peninsula (Bradshaw & Brook 2007). These events would have increased the fluctuations in N_e expected under normal environmental and demographic stochasticity, with the added possibility of reducing N_e even more via male-biased hunting for safari (Brook *et al*. 2006; Bradshaw & Brook 2007). Our successful prediction highlights the utility of combining basic age-structured population models with theoretical expectations of genetic diversity to investigate the potential impacts of genetic bottlenecks. We suggest that such models can be used as tools to infer the expected genetic variation in vulnerable and range-restricted species in the absence of ideal source population data and observable levels of genetic diversity.

Could animals from Australia be re-introduced to Southeast Asia (Corbett 1995) to increase the overall genetic variability of the native inbred remnants (i.e. genetic 'rescue'; Ingvarsson 2001)? Given that Australian banteng are pure-strain *B. javanicus* (Bradshaw *et al*. 2006), they could lower inbreeding and add genetic diversity to small wild Asian banteng populations. The most serious risk involved here is that it may lead to outbreeding depression if banteng in Australia have adapted to local conditions that are suboptimal in their Southeast Asian range (e.g. Greig 1979). Some similarities in these two tropical environments suggest that this may not be a large risk, and natural selection should alleviate any initial problems with time. The Australian population has a domesticated origin (Bradshaw *et al*. 2006), so the source population may have experienced selection for traits deemed desirable for domestic stock. However, 150 years of wild reproduction should have minimized any deleterious consequences of this. Further, it is possible that a re-introduction of Australian banteng to Southeast Asia could inadvertently introduce Australian diseases or parasites (e.g. Ross River virus, Japanese encephalitis), but this should be avoidable with careful veterinary testing and quarantine procedures. Overall however, there exists relatively little convincing evidence that such concerns about re-introductions would outweigh the fitness benefits of reduced inbreeding and increased genetic diversity (Frankham *et al*. 2002). Although the number of wild banteng sampled from their native range was low ($n = 4$), the lower genetic diversity relative to domesticated Bali cattle is consistent with the prediction that the endangered populations of *B. javanicus* in their native range have suffered a genetic bottleneck, thus potentially warranting genetic augmentation from re-introductions.

The low genetic variability observed in Australian banteng has not stymied its capacity to recover from a founder bottleneck, or for it to support a sustainable harvest (Bradshaw & Brook 2007). However, inbreeding depression can be insidious, leading, for instance, to higher parasite loads (Coltman *et al*. 1999) or a reduced capacity to withstand severe environmental extremes (Keller *et al*. 2002). Furthermore, ill-conceived control programmes of the Australian herd or over-harvesting could potentially impact adversely the genetic integrity of the population. Although the genetic management of the manifestly inbred Australian banteng is not yet a conservation priority, it may become one in the future as the remaining wild banteng in Southeast Asia are possibly driven to extinction. In this context, this accident of a historical introduction may prove to be essential for the (*ex situ*) survival of this species. Our results indicate that wild populations of some endangered species can be established successfully *ex situ* in spite of reduced genetic variability — a situation that may become more common as intact habitats worldwide become increasingly destroyed and degraded. The future of many wild megafauna species may therefore hinge on their ability to persist in spite of severe genetic erosion.

Acknowledgements

We thank J. A. Lenstra, O. Hanotte and I. J. Nijman for providing reference data for Bali cattle and wild-caught banteng. This project was funded by the Australian Research Council (Linkage Grant LP0349305), the Parks and Wildlife Service of the Northern Territory, Charles Darwin University, and Grants-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology of Japan (No. 14405006). J. A. Lenstra and four anonymous reviewers provided helpful comments to improve the manuscript. All capture and sampling procedures were approved by the Charles Darwin Animal Ethics Committee and authorized under a Parks and Wildlife Service of the Northern Territory Permit to Take Wildlife for Commercial Purposes (No. 18275). Logistics and fieldwork were facilitated by P. Butler, J. Christophersen, P. Cooper, R. Cunningham, P. Fitzgerald, D. Scammell, L. Traill, J. Wauchope, K. Wauchope, K. Wertz, W. White, G. Williams, and J. Williams. We also thank the Territory Wildlife Park (Berry Springs, Northern Territory) for access to captive animals.

References

Amos W, Balmford A (2001) When does conservation genetics matter? *Heredity*, **87**, 257–265.

Armbruster P, Reed DH (2005) Inbreeding depression in benign and stressful environments. *Heredity*, **95**, 235–242.

Balloux F, Amos W, Coulson T (2004) Does heterozygosity estimate inbreeding in real populations? *Molecular Ecology*, **13**, 3021–3031.

Barker JSF, Moore SS, Hetzel DJS *et al*. (1997a) Genetic diversity of Asian water buffalo (*Bubalus bubalis*): microsatellite variation and a comparison with protein-coding loci. *Animal Genetics*, **28**, 103–115.

Barker JSF, Tan SG, Selvaraj OS, Mukherjee TK (1997b) Genetic variation within and relationships among populations of Asian water buffalo (*Bubalus bubalis*). *Animal Genetics*, **28**, 1–13.

Beja-Pereira A, Zeyl E, Ouragh L *et al*. (2004) Twenty polymorphic microsatellites in two of North Africa's most threatened ungulates: *Gazella dorcas* and *Ammotragus lervia* (Bovidae; Artiodactyla). *Molecular Ecology Notes*, **4**, 452–455.

Berger J, Cunningham C (1995) Multiple bottlenecks, allopatric lineages and badlands bison *Bos bison* — consequences of lineage mixing. *Biological Conservation*, **71**, 13–23.

Bessa-Gomes C, Legendre S, Clobert J (2004) Allee effects, mating systems and the extinction risk in populations with two sexes. *Ecology Letters*, **7**, 802–812.

Borlase SC, Loebel DA, Frankham R *et al*. (1993) Modeling problems in conservation genetics using captive *Drosophila* populations: consequences of equalization of family sizes. *Conservation Biology*, **7**, 122–131. *Conservation Biology*, **7**, 122–131.

Bradshaw CJA, Brook BW (2007) Ecological-economic models of sustainable harvest for an endangered but exotic megaherbivore in northern Australia. *Natural Resource Modeling*, **20**, 129–156.

Bradshaw CJA, Traill LW, Wertz KL, White WH, Gurry IM (2005) Chemical immobilisation of wild banteng (*Bos javanicus*) in northern Australia using detomidine, tiletamine and zolazepam. *Australian Veterinary Journal*, **83**, 616–617.

Bradshaw CJA, Isagi Y, Kaneko S, Bowman DMJS, Brook BW (2006) Conservation value of non-native banteng in northern Australia. *Conservation Biology*, **20**, 1306–1311.

Brezinsky L, Kemp SJ, Teale AJ (1993) ILSTS6: a polymorphic bovine microsatellite. *Animal Genetics*, **24**, 73.

Broders HG, Mahoney SP, Montevecchi WA, Davidson WW (1999) Population genetic structure and the effect of founder events on the genetic variability of moose, *Alces alces*, in Canada. *Molecular Ecology*, **8**, 1309–1315.

Brook BW, Bowman DMJS, Bradshaw CJA, Campbell BM, Whitehead PJ (2006) Managing an endangered Asian bovid in an Australian national park: the role and limitations of ecological-economic models in decision-making. *Environmental Management*, **38**, 463–469.

Caballero A (1994) Developments in the prediction of effective population size. *Heredity*, **73**, 657–679.

Caro TM, Laurenson MK (1994) Ecological and genetic factors in conservation: a cautionary tale. *Science*, **263**, 485–486.

Caswell H (1989) *Matrix Population Models: Construction, Analysis, and Interpretation*. Sinauer & Associates, Sunderland, Massachusetts.

Caughley G (1994) Directions in conservation biology. *Journal of Animal Ecology*, **63**, 215–244.

Ceballos G, Ehrlich PR (2002) Mammal population losses and the extinction crisis. *Science*, **296**, 904–907.

Ciofi C, Bruford MW (1999) Genetic structure and gene flow among Komodo dragon populations inferred by microsatellite loci analysis. *Molecular Ecology*, **8**, S17–S30.

Coltman DW, Pilkington JG, Smith JA, Pemberton JM (1999) Parasite-mediated selection against inbred Soay sheep in a free-living, island population. *Evolution*, **53**, 1259–1267.

Corbett LK (1995) Bali banteng. In: *The Mammals of Australia* (ed. Strahan R), pp. 726–728. Reed New Holland, Sydney.

DeYoung RW, Demarais S, Honeycutt RL *et al*. (2003) Genetic consequences of white-tailed deer (*Odocoileus virginianus*) restoration in Missippi. *Molecular Ecology*, **12**, 3237–3252.

Eldridge MDB, King JM, Loupis AK *et al*. (1999) Unprecedented low levels of genetic variation and inbreeding depression in an island population of the black-footed rock-wallaby. *Conservation Biology*, **13**, 531–541.

Engen S, Lande R, Saether B-E (2005) Effective size of a fluctuating age-structured population. *Genetics*, **170**, 941–954.

European Cattle Genetic Diversity Consortium (2006) Marker-assisted conservation of European cattle breeds: an evaluation. *Animal Genetics*, **37**, 475–481.

Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics*, **131**, 79–491.

Forbes SH, Hogg JT, Buchanan FC, Crawford AM, Allendorf FW (1995) Microsatellite evolution in congeneric mammals: domestic and bighorn sheep. *Molecular Biology and Evolution*, **12**, 1106–1113.

Frankel OH, Soulé ME (1981) *Conservation and Evolution*. Cambridge University Press, Cambridge, UK.

Frankham R (1995) Effective population size/adult population size ratios in wildlife: a review. *Genetical Research*, **66**, 95–107.

Frankham R (2005) Genetics and extinction. *Biological Conservation*, **126**, 131–140.

Frankham R, Ballou JD, Briscoe DA (2002) *Introduction to Conservation Genetics*. Cambridge University Press, Cambridge, UK.

Goodman SJ, Tamate HB, Wilson R *et al*. (2001) Bottlenecks, drift and differentiation: the population structure and demographic history of sika deer (*Cervus nippon*) in the Japanese archipelago. *Molecular Ecology*, **10**, 1357–1370.

Goudet J (1992) FSTAT (version 1.2): a computer program to calculate *F*-statistics. *Journal of Heredity*, **86**, 485–486.

Greig JC (1979) Principles of genetic conservation in relation to wildlife management in Southern Africa. *South African Journal of Wildlife Research*, **9**, 57–78.

Hedges S (1996) Proposal for Inclusion of Banteng (*Bos Javanicus*) in CITES Appendix I. IUCN/SSC Asian Wild Cattle Specialist Group, IUCN/SSC Wildlife Trade Programme, Thai Government, Bangkok.

Hmwe SS, Zachos FE, Eckert I *et al*. (2006) Conservation genetics of the endangered red deer from Sardinia and Mesola with further remarks on the phylogeography of *Cervus elaphus corsicanus*. *Biological Journal of the Linnean Society*, **88**, 691–701.

Holm LE, Forchhammer MC, Boomsma JJ (1999) Low genetic variation in muskoxen (*Ovibos moschatus*) from western Greenland using microsatellites. *Molecular Ecology*, **8**, 675–679.

Ingvarsson PK (2001) Restoration of genetic variation lost — the genetic rescue hypothesis. *Trends in Ecology & Evolution*, **16**, 62–63.

IUCN (2005) *IUCN Red List of Threatened Species*. World Conservation Union, Gland, Switzerland. Available at URL: www.iucnredlist.org

Jepsen BI, Siegismund HR, Fredholm M (2002) Population genetics of the native caribou (*Rangifer tarandus groenlandicus*) and the semi-domestic reindeer (*Rangifer tarandus tarandus*) in Southwestern Greenland: evidence of introgression. *Conservation Genetics*, **3**, 401–409.

Keller LF, Waller DM (2002) Inbreeding effects in wild populations. *Trends in Ecology & Evolution*, **17**, 230–241.
Keller LF, Arcese P, Smith JNM, Hochachka WM, Stearns SC (2002) Selection against inbred song sparrows during a natural population bottleneck. *Nature*, **372**, 356–357.
Kemp SJ, Hishida O, Wambugu J *et al.* (1995) A panel of polymorphic bovine, ovine and caprine microsatellite markers. *Animal Genetics*, **26**, 299–306.
Kirby GWM (1979) Bali cattle in Australia. *FAO World Animal Review*, **31**, 1–7.
Lande R (1988) Genetics and demography in biological conservation. *Science*, **241**, 1455–1460.
Le Page SL, Livermore RA, Cooper DW, Taylor AC (2000) Genetic analysis of a documented population bottleneck: introduced Bennett's wallabies (*Macropus rufogriseus rufogriseus*) in New Zealand. *Molecular Ecology*, **9**, 753–763.
Leberg PL (2002) Estimating allelic richness: effects of sample size and bottlenecks. *Molecular Ecology*, **11**, 2445–2449.
Letts GA (1964) Feral animals in the Northern Territory. *Australian Veterinary Journal*, **40**, 84–88.
Lomolino MV, Channell R (1998) Range collapse, re-introductions, and biogeographic guidelines for conservation. *Conservation Biology*, **12**, 481–484.
Maudet C, Beja-Pereira A, Zeyl E *et al.* (2004) A standard set of polymorphic microsatellites for threatened mountain ungulates (Caprini, Artiodactyla). *Molecular Ecology Notes*, **4**, 49–55.
Moritz C (1999) Conservation units and translocations: strategies for conserving evolutionary processes. *Hereditas*, **130**, 217–228.
Nei M (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics*, **89**, 583–590.
Nei M, Maruyama T, Chakraborty R (1975) The bottleneck effect and genetic variability in populations. *Evolution*, **29**, 1–10.
Newman D, Pilson D (1997) Increased probability of extinction due to decreased genetic effective population size: experimental populations of *Clarkia pulchella*. *Evolution*, **51**, 354–362.
Nijman IJ, Otsen M, Verkaar EL *et al.* (2003) Hybridization of banteng (*Bos javanicus*) and zebu (*Bos indicus*) revealed by mitochondrial DNA, satellite DNA, AFLP and microsatellites. *Heredity*, **90**, 10–16.
O'Grady JJ, Brook BW, Reed DH *et al.* (2006) Realistic levels of inbreeding depression strongly affect extinction risk in wild populations. *Biological Conservation*, **133**, 42–51.
O'Ryan C, Harley EH, Bruford MW *et al.* (1998) Microsatellite analysis of genetic diversity in fragmented South African buffalo populations. *Animal Conservation*, **1**, 85–94.
Paetkau D, Strobeck C (1994) Microsatellite analysis of genetic variation in black bear populations. *Molecular Ecology*, **3**, 489–495.
Paterson S, Pemberton JM (1997) No evidence for major histocompatibility complex-dependent mating patterns in a free-living ruminant population. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **264**, 1813–1819.
Peakall R, Smouse PE (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes*, **6**, 288–295.
Rice KJ, Emery NC (2003) Managing microevolution: restoration in the face of global change. *Frontiers in Ecology and the Environment*, **1**, 469–478.
Saccheri I, Kuussaari M, Kankare M *et al.* (1998) Inbreeding and extinction in a butterfly metapopulation. *Nature*, **392**, 491–494.
Sambrook J, Russell WD (2001) *Molecular Cloning: a Laboratory Manual*, 3rd edn. Cold Spring Harbor Press, Cold Spring Harbor, NY.
Slate J, Coltman DW, Goodman SJ *et al.* (1998) Bovine microsatellite loci are highly conserved in red deer (*Cervus elaphus*), sika deer (*Cervus nippon*) and Soay sheep (*Ovis aries*). *Animal Genetics*, **29**, 307–315.
Spielman D, Brook BW, Frankham R (2004) Most species are not driven to extinction before genetic factors impact them. *Proceedings of the National Academy of Sciences, USA*, **101**, 15261–15264.
Tapio I, Värv S, Bennewitz J *et al.* (2006) Prioritization for conservation of northern European cattle breeds based on analysis of microsatellite data. *Conservation Biology*, **20**, 1768–1779.
Taylor AC, Sherwin WB, Wayne RK (1994) Genetic variation of microsatellite loci in a bottlenecked species — the northern hairy-nosed wombat *Lasiorhinus krefftii*. *Molecular Ecology*, **3**, 277–290.
Taylor AC, Horsup A, Johnson CN, Sunnucks P, Sherwin WB (1997) Relatedness structure detected by microsatellite analysis and attempted pedigree reconstruction in an endangered marsupial, the northern hairy-nosed wombat (*Lasiorhinus krefftii*). *Molecular Ecology*, **6**, 9–19.
Thomas CD, Cameron A, Green RE *et al.* (2004) Extinction risk from climate change. *Nature*, **427**, 145–148.
Van Hooft WF, Groen AF, Prins HHT (2000) Microsatellite analysis of genetic diversity in African buffalo (*Syncerus caffer*) populations throughout Africa. *Molecular Ecology*, **9**, 2017–2025.
Visscher PM, Smith D, Hall SJG, Williams JL (2001) A viable herd of genetically uniform cattle. *Nature*, **409**, 303.
Williams LW, Serfass TL, Cogan R, Rhodes OE Jr (2002) Microsatellite variation in the reintroduced Pennsylvania elk herd. *Molecular Ecology*, **11**, 1299–1310.
Wilson GA, Strobeck C (1999) Genetic variation within and relatedness among wood and plains bison populations. *Genome*, **42**, 483–496.
Wilson GA, Nishi JS, Elkin BT, Strobeck C (2005) Effects of a recent founding event and intrinsic population dynamics on genetic diversity in an ungulate population. *Conservation Genetics*, **6**, 905–916.
Wright S (1931) Evolution in Mendelian populations. *Genetics*, **16**, 97–159.
Wright S (1969) *Evolution and the Genetics of Populations*. University of Chicago Press, Chicago.

CJAB collected the data, did the statistical analyses and took the lead in writing the paper. YI and SK did the genetic analyses and some of the statistical analyses. BWB and RF contributed to the population modelling, and DMJSB, CJAB and BWB jointly conceived the work. All coauthors contributed to the writing of the paper.
