Conservation Value of Non-Native Banteng in Northern Australia

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Abstract: *The global species extinction crisis has provided the impetus for elaborate translocation, captive breeding, and cloning programs, but more extreme actions may be necessary. We used mitochondrial DNA, Y-chromosome, and nuclear lactoferrin-encoding gene sequencing to identify a wild population of a pure-strain endangered bovid (*Bos javanicus*) introduced into northern Australia over 150 years ago. This places the Australian population in a different conservation category relative to its domesticated conspecific in Indonesia (i.e., Bali cattle) that has varying degrees of introgression from other domesticated* Bos *spp. The success of this endangered non-native species demonstrates that although risky, the deliberate introduction of threatened exotic species into non-native habitat may provide, under some circumstances, a biologically feasible option for conserving large herbivores otherwise imperiled in their native range.*

Keywords: *Bos javanicus*, endangered species, translocation, non-native species, semidomestication

Valor de Conservación de *Bos javanicus* Introducido en el Norte de Australia

Resumen: *La crisis global de extinción ha proporcionado el ímpetu para elaborar programas de translocación, reproducción en cautiverio y clonación, pero es probable que se requieran acciones más extremas. Mediante la utilización de ADN mitocondrial, cromosoma-Y y secuenciación del gene codificador de lactoferrina, reportamos la identificación de una población silvestre de una cepa pura del bóvido en peligro (*Bos javanicus*) introducido en el norte de Australia hace más de 150 años. Esto coloca a la población australiana en una categoría de conservación diferente a la de su conespecífica en Indonesia (i.e., ganado de Bali) que tiene diferentes grados de introgresión de otras* Boss *spp. domesticadas. El éxito de esta especie no nativa en peligro demuestra que, aunque arriesgada, la introducción de especies exóticas amenazadas puede proporcionar, bajo ciertas circunstancias, una opción biológicamente factible para la conservación de herbívoros en peligro en su hábitat nativo.*

Palabras Clave: *Bos javanicus*, especies en peligro, especies no nativas, semidomesticación, translocación

Introduction

Extreme actions may be required to preserve viable populations of wide-ranging, large-bodied vertebrates threatened by habitat loss, overexploitation, and climate change. Conservation options could include careful, deliberate introduction to similar habitats outside their native ranges (Bowman 1993; Martin & Burney 2000; Donlan et al. 2005), especially considering accelerated global environmental change (Peters 1992). Critics of this approach warn of the potential disadvantages for host regions, including habitat degradation, altered community dynamics, disease introduction, uncontrolled pest proliferation, and the cost of failed efforts because of overly

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specific habitat requirements and inbreeding depression (Gipps 1991; Donlan et al. 2005). However, the potential global conservation benefits are also high, especially if ex situ populations of threatened species can be established to reduce their probability of extinction.

Australia lost most of its endemic megafauna during the late Pleistocene (Brook & Bowman 2004), but now hosts extensive populations of many large-bodied exotic mammalian species: Eurasian wild pigs (*Sus scrofa*), camels (*Camelus dromedarius*), horses (*Equus caballus*), donkeys (*E. asinus*), chital (*Axis axis*), hog deer (*A. porcinus*), rusa (*Cervus timorensis*), red deer (*C. elaphus*), sambar deer (*C. unicolor*), fallow deer (*Dama dama*), swamp buffalo (*Bubalus bubalis*), and hybrid or possibly pure-strain banteng (*Bos javanicus*) (Freeland 1990; Strahan 1995). Of these, only banteng and swamp buffalo are listed as endangered (IUCN 2005), although it is debatable whether any purebred wild buffalo remain even in their native range (Hedges 1996*b*). There has been a recent, partially successful (although expensive) attempt to clone banteng from captive specimens (BBC 2003).

In 1849, approximately 20 domesticated banteng from Denpasar, Bali, were introduced to Port Essington on an isolated peninsula in western Arnhem Land and subsequently became wild after the settlement's failure in the same year (Calaby 1975; Corbett 1995). Banteng have remained on the peninsula (220000 ha), and the population has grown to ~6000 (K. Saalfeld, unpublished data). The population these original animals generated has remained wild with absolutely no human control for over 150 years. The genetic purity of the relatively abundant and domesticated banteng (henceforth called Bali cattle to differentiate them from their wild, pure-strain counterparts, banteng) within Southeast Asia is doubtful based on genetic evidence and phenotypic characteristics resulting from indiscriminate crossbreeding with other *Bos* spp. (Namikawa 1981; Verkaar et al. 2002; Handiwirawan et al. 2003; Nijman et al. 2003; IUCN 2005). Thus, the conservation and trade status of the Australian banteng under CITES criteria has been ambiguous, despite the suggestion that this population represents a potential genetic reservoir for the species (Corbett 1995). Nonetheless, safari hunters from around the world pay substantial money for the privilege of shooting some (<150/year) of these wild, albeit non-native (and possibly endangered) bovids (Corbett 1995).

Genetic analyses are able to clarify the taxonomic identity of banteng in Australia, and they also raise profound issues that demand a judicious management response. If Australian banteng have genetic introgression from congeners, this population is little more than an unusual variety of feral cattle. However, if they are pure-strain *B. javanicus*, then the appellation *feral* may be inappropriate because it ignores the countervailing endangered status of the remaining wild (i.e., free-ranging and undomesticated) banteng within their native range, and it generally has negative connotations with respect to biodiversity conservation. Under existing conservation policies, the extermination or commercial exploitation of feral species may be easier to justify than if they are also considered endangered. Therefore, we analyzed the mitochondrial and nuclear DNA from wild-sampled Australian banteng to establish their true genetic identity given the uncertainty in their status. We also considered the Australian and international legislative implications of identifying a non-native species as endangered, and the issue of intentionally establishing other ex situ populations of endangered species in Australia for commercial and conservation purposes (Donlan et al. 2005).

Methods

Data Collection

Fifty-four free-ranging banteng from Garig Gunak Barlu National Park, Cobourg Peninsula, Northern Territory, Australia (11° 20′ S, 132° 20′ E, Fig. 1) were sampled for skin tissue with two methods: (1) pursuit on foot followed by shooting a biopsy dart from a 22 calibre, cartridge-fired dart projector (Pneu-Dart, Williamsport, Pennsylvania),

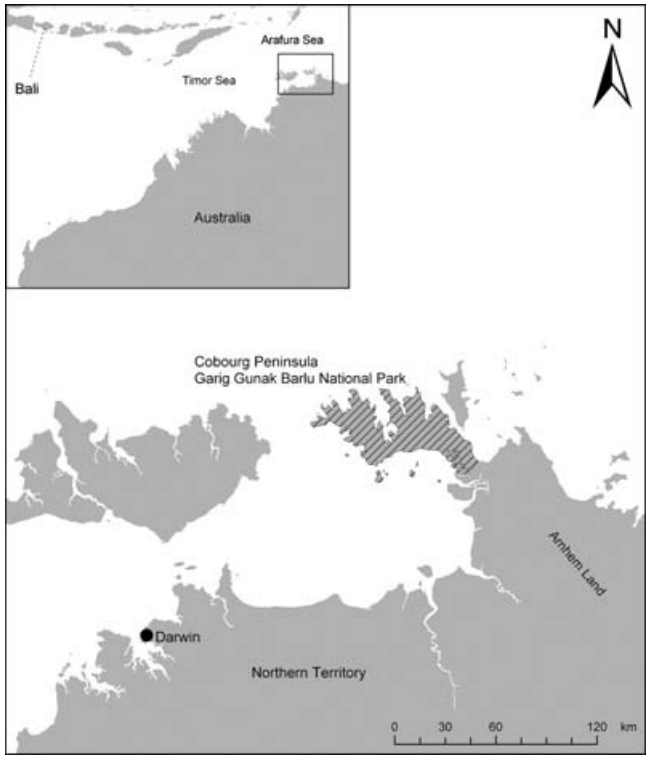

Figure 1. Location of the Australian banteng population in Garig Gunak Barlu National Park on the Cobourg Peninsula, Northern Territory, relative to their point of origin in Bali, Indonesia.

or (2) direct capture via remote delivery of chemical immobilization followed by manual biopsy of the skin (Bradshaw et al. 2005). We collected samples from male ($n_m = 41$) and female ($n_f = 13$), and juvenile ($n_j = 6$) and adult ($n_a = 48$) animals from four distinct areas within the park separated by 40–75 km ($n_1 = 24$, $n_2 = 20$, $n_3 = 3$, $n_4 = 7$, respectively). Skin samples were stored in 90% ethanol.

DNA Extraction and Analysis

We used standard SDS/proteinase K protocol and phenol/chloroform procedures, respectively to extract and purify DNA (Sambrook & Russell 2001). Mitochondrial DNA (mtDNA) cytochrome c oxidase subunit II (COII) gene (Janecek et al. 1996), nuclear male-specific gene *SRY* (Kikkawa et al. 2003), and noncoding regions from a nuclear gene, the promoter segment of the lactoferrin-encoding gene (Hassanin & Douzery 1999), were amplified by PCR and then sequenced. We amplified the mtDNA COII region with *TaKaRa* EX taq according to the supplier's protocol (*TaKaRa* Shuzo Co., Otsu, Japan). The PCR conditions were an initial denaturation at 94° C for 2 minutes, followed by 35 cycles at 95° C for 1 minute, 45° C for 1 minute, and 72° C for 1.25 minutes. For the nuclear genes, *SRY*, and the promoter segment of the lactoferrin-encoding gene, we performed amplifications with AmpliTaq Gold (Applied Biosystems, Foster City, California), with an initial denaturation at 95° C for 10 minutes, followed by 35 cycles at 94° C for 30 seconds, annealing for 30 seconds, and 72° C for 2 minutes. The annealing temperatures were 50° C for *SRY* and 61° C for the promoter segment of the lactoferrin-encoding gene. The amplified DNA fragments were subjected to electrophoresis on a 1.5% agarose gel and then purified with High Pure PCR Product Purification Kit (Roche Applied Science, Nonnenwald, Penzberg, Germany). The purified products were sequenced directly with ABI BigDye Terminators Cycle Sequencing Kit (version 3.1) on an ABI PRISM 3100 Genetic Analyzer. Electropherograms were assembled with Sequencher 3.1 software (GeneCodes, Ann Arbor, Michigan). We compared aligned sequences of each locus with those obtained from GenBank for pure-strain *B. javanicus* (accession nos. U18821, AB077319, and AY689192), *B. taurus* (accession nos. NC001567, U15569, and AF281088), and *B. indicus* (accession nos. U18820, AB077318, and AY689193).

Results

All sequences analyzed for the 54 animals (586 bps for COII, 506 bps for *SRY*, and 327 bps for the promoter segment of the lactoferrin-encoding gene) were completely consistent with that of *B. javanicus* and inconsistent with that of *B. taurus* (domestic cattle) or *B. indicus* (zebu) at 28 sites in the locus COII and one site in the locus *SRY*. The gene sequences analyzed here were shorter than those reported by Kikkawa et al. (2003) for the *SRY* gene, by Janecek et al. (1996) for the COII gene, and by Hassanin and Douzery (1999) for the lactoferrin-encoding gene (690, 684, and 338 bps, respectively). This is due to the observation that the two margins of the target region are often obscure, so we omitted these to provide a shorter sequence sufficient for the identification of *B. javanicus*.

The genetic results are also consistent with our observations that all sampled individuals demonstrated morphological characteristics of banteng (e.g., short, black/dark brown pelage for males, reddish/brown pelage for females, pronounced white rump patch and leggings, white muzzle) and not feral *B. taurus* or *B. indicus*. Furthermore, there is no evidence of reduced body size in the Australian population relative to free-ranging banteng in their native range given that many trophy males have been shot in Australia that are comparable in size to Asian-harvested individuals (Safari Club International 2003). Thus, we conclude that Australian banteng are pure-strain *B. javanicus*. These findings are further supported by the known relationships between the species examined. Recent phylogenies constructed for the Bovinae indicate that *B. javanicus* forms a separate clade from *B. taurus* and *B. indicus*. Buntjer et al. (2002) found that *B. javanicus* formed a grouping with gaur (*B. gaurus*) and not with *B. taurus* or *B. indicus* based on amplified fragment-length polymorphism. However, the analysis assigned inconsistent positions of *B. javanicus* within the phylogeny. This is consistent with the results of Janecek et al. (1996), who found a closer affinity between *B. gaurus* and *B. javanicus* than to *B. taurus* based on the COII gene, although the average percentage of nucleotide sequence divergence that separated the three species was 5.1% (compared with only 0.6% between *B. taurus* and yak, *B. grunniens*). Kikkawa et al. (2003) also found that *B. javanicus* represents a separate clade to *B. indicus* based on mtDNA. Although some of the sites found to be phylogenetically informative may be polymorphic, the magnitude of the sequence divergence observed in our samples suggests that it is unlikely to alter our conclusions.

We were unable to detect any polymorphisms in the *SRY*, COII, or lactoferrin-encoding gene sequences for the samples obtained. The lack of polymorphisms is likely to be a function of the severe bottleneck experienced by this population with the release of only 20 individuals into the Cobourg Peninsula in 1849. This suggests that heterozygosity in this population may be low but cannot be quantified explicitly with the analysis of the genes used for species identification. Additional comparisons of wild *B. javanicus* in their native range in Southeast Asia with the Australian population of *B. javanicus* may show the

degree of heterozygosity present in the Cobourg Peninsula population based on other microsatellite loci.

Discussion

Today in Asia, there are fewer than 5000 wild, pure-strain *B. javanicus* divided among small (<500 individuals), disjunct populations (Hedges 1996*a*; IUCN 2005). Their range has declined by 85% in the last 15–20 years, and the most important contemporary threats to this species are hunting, habitat loss, and interbreeding with domestic and feral cattle species (Hedges 1996*a*; IUCN 2005). Australian banteng therefore represent a potentially important reservoir for the wild form of this species. The potentially reduced heterozygosity may limit this role, although many threatened species of native ungulates appear to retain moderate to high levels of genetic variability even at low population sizes (e.g., native cattle breeds in Europe [Kantanen et al. 1999; Rendo et al. 2004], Vietnamese sika deer [*Cervus nippon pseudaxis*] [Thevenon et al. 2004], Swayne's hartebeest [*Alcelaphus buselaphus swaynei*] in Ethiopia [Flagstad et al. 2000], and Sumatran rhinoceros [*Dicerorhinus sumatrensis*] [Scott et al. 2004]). Indeed, the rapid population growth and stabilization demonstrated for the Australian population of banteng (Bradshaw & Brook 2007) is testament to the lack of reduction in demographic rates due to possible low heterozygosity.

Genetic introgression from species *A* to species *B* can result in alleles from *A* being found in the autosomes of *B* (Harrison 1993; Avise 2004), and when this occurs, it is usually expected that specific alleles from *A* will also be found in maternally inherited mtDNA and/or the paternally inherited *Y* chromosome of species *B*. Furthermore, the ratio of species *B* individuals with alleles from species *A* to all individuals in the population varies relative to the degree of introgression. We did not detect any alleles of either *B. indicus* or *B. taurus* in the mtDNA or *Y* chromosomes of the 54 Australian banteng sampled (approximately 0.9% of the population). The consistency of the biparentally inherited locus of the lactoferrin-encoding gene with *B. javanicus* is important because using only sex-specific genes produces the chance of detecting homozygous genotypes diagnostic for one parental type even though the individual is a hybrid. These three lines of evidence indicate clearly that the Australian banteng population consists of genetically pure *B. javanicus*.

The importance of this distinction between wild and domesticated populations is critical. First, most domesticated Bali cattle throughout their range appear to have some degree of genetic introgression from other *Bos* species (Namikawa 1981; Verkaar et al. 2002; Handiwirawan et al. 2003; Nijman et al. 2003; Verkaar et al. 2003) and do not form contiguous interbreeding populations of pure individuals. So the possibility for continued hybridization is high, except perhaps on the Island of Bali, where strict cattle importation laws are in place (Martojo 2003). Even though a Y-chromosomal analysis of five domesticated Bali cattle bulls from Bali demonstrated that four of them appeared to descend from banteng bulls (Verkaar et al. 2003), these animals are still domesticated and therefore are of a lower conservation value than the pure strain, wild population in Australia.

If managers consider Australian banteng pest animals and not a wild, endangered species surviving ex situ, there is a possibility they will elect an eradication program to reduce their densities or to eliminate them entirely from Australia (as was attempted for swamp buffalo. [Robinson & Whitehead 2003]). If Australian banteng are eliminated, then there is a high risk that all wild individuals of this species will become extinct in the near future because the threats to them in Southeast Asia have not abated (IUCN 2005). Furthermore, the observation that most domesticated Bali cattle have some form of genetic introgression strongly suggests that there is also the threat of losing the genetically pure form of this species through continued hybridization. We therefore urge managers to consider the conservation status of Australian banteng carefully in light of our findings.

Our results for Australian banteng will now have to be considered by international (e.g., World Conservation Union, CITES, Convention on Biological Diversity) and Australian authorities with regard to the species' conservation status. Although the legal and political context for ex situ conservation (defined as "the conservation of components of biological diversity outside their natural habitats," Glowka et al. 1994) has changed sufficiently to recognize it as a valid conservation tool (Maunder & Byers 2005), there appears to be no provision in the Convention on Biological Diversity for the deliberate introduction and establishment of wild (uncontained) populations of exotic animals for conservation purposes. Clearly, the acceptance of current and future introductions of exotic species as a valid component of ex situ conservation requires modification to existing legislation.

If one accepts that Australian *B. javanicus* represents a distinct category of this species (i.e., wild, pure strain but ex situ), our findings illustrate how a highly endangered, large-bodied species can be buffered from extinction by its successful establishment in non-native areas. Although it has been suggested that introduced banteng have had some negative impacts on coastal grasslands through overgrazing and on small mammal burrows through trampling (Corbett 1995), the observed damage is considerably less than that caused by other non-native and feral species in the region such as pigs and buffalo (Bowman & Panton 1991; Corbett 1995). The successful establishment of Australian banteng was largely accidental, but it does provide a real-world example of how successful introductions of threatened, large-bodied species outside

their native range can be achieved (see Bowman 1993; Martin & Burney 2000; Donlan et al. 2005).

Akin to the introduction of non-native, large-bodied species to North America recently advocated by Donlan et al. (2005), many endangered and critically endangered (IUCN 2005) artiodactyl and perissodactyl species are potential candidates for introduction to Australia because as herbivores, they would not impose any predation pressure on indigenous fauna. Indeed, many arid (e.g., African) and tropical (e.g., Asian) specialists requiring large areas may be particularly suitable, and these could include the addax (*Addax nasomaculatus*), Walia ibex (*Capra walie*), rhim (*Gazella leptoceros*), hirola (*Damaliscus hunteri*), scimitar-horned oryx (*Oryx dammah*), and Grevy's zebra (*E. grevyi*) of Africa, the anoa (*Bubalus depressicornis*), tamaraw (*B. mindorensis*), pygmy hog (*S. salvanius*), Javan warty pig (*S. verrucosus*), Philippine spotted deer (*Cervus alfredi*), calamian deer (*Axis calamianensis*), and bawean deer (*Axis kuhlii*) of Southeast Asia, and the Chacoan peccary (*Catagonus wagneri*) of South America. Our results also suggest that the conservation status of other "feral" animal species that have established larger wild populations in Australia than those occurring in their native range (e.g., swamp buffalo and camels) might have to be reconsidered.

The survival of large, endangered species such as banteng may ultimately hinge on their establishment in nonendemic regions, effectively operating as vast open-range zoos. The success of such approaches depends on the suitability of the ex situ habitat to sustain the species in question and the ability to introduce the required minimum number of individuals to ensure long-term population persistence and sufficient genetic diversity (Amos & Balmford 2001). Methods used to optimize the probability of reintroduction success via translocation (Griffith et al. 1989) can also be applied to the establishment of ex situ populations. Clearly, assessments of establishment potential, negative impacts on indigenous wildlife, disease screening, and the capacity to contain exotic species to specific areas would be essential for this approach to be successful (Donlan et al. 2005).

The suggestion that other exotic species should be considered as candidates for introduction to countries such as Australia to buffer populations from extinction is controversial (Donlan et al. 2005), and we acknowledge this openly. However, we argue that the global conservation crisis requires extreme actions to offset species' extinction rates. This "semidomestication" for conservation purposes is admittedly an extreme approach, yet it may provide a legitimate alternative to extensive cattle ranching worldwide (e.g., replacing revenue derived from cattle ranching with that earned by tourism viewing or safari-type harvests of wild exotic fauna, Bowman 1993; O'Rourke 2000). Nonetheless, in certain circumstances, and provided conservation managers proceed with extreme caution, it should be possible to make deliberate, managed introductions of other endangered exotics without threatening indigenous biodiversity seriously. This action is comparable to intensively managed game parks within the native range of the world's surviving megafauna, where wildlife species are effectively in a state of semidomestication already (Armbruster & Lande 1993; Neumann 2001; Donlan et al. 2005). Thus, the notion of truly wild megafauna may be as endangered as the species themselves. If the decision was made to release endangered exotic species, we suggest that the target ecosystem be monitored prior to and after release and that monitoring plans be developed within conservation schemes prior to release.

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