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Genetic structure of introduced swamp buffalo subpopulations in tropical Australia

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Abstract High densities of introduced herbivores can damage sensitive ecosystems, increase the risk of extinction of native biota, and host and spread disease. An essential step in managing large 'feral' animal populations is to quantify how they use habitats so that management interventions, such as culling, can be targeted to reduce densities and to minimize migration into areas from which animals have been removed. An effective method to quantify animal movements is by measuring landscape-scale genetic population structure. We describe the genetic population structure of one of Australia's more destructive introduced mammals – the Asian swamp buffalo (*Bubalus bubalis*). We collected 524 skin samples from buffalo across their range in the Northern Territory of Australia. Allelic diversity in the Northern Territory population was low compared to those reported from populations in their native Asian habitats. The Australian population is tentatively made of three subpopulations; Melville Island, Eastern Arnhem and Central-Western Arnhem populations. The Melville Island population is represented by a single cluster, while the Eastern Arnhem population has three clusters and the Central-Western Arnhem population seven clusters. We found some support for isolation by distance across all the sampled populations, but little evidence for this relationship when comparing the two well-mixed mainland metapopulations. Despite their small founder populations and limited genetic variation, the persistence of buffalo in Australia has likely been aided by release from high predation, parasitism and disease typical of their native habitats.

Key words: *Bubalus bubalis*, culling, feral animal, founder effect, genetic diversity, heterozygosity, management, migration.

INTRODUCTION

Invasive species are important drivers of biodiversity loss because they can damage sensitive ecosystems and native biota, and they act as hosts of diseases that are transmissible to other species including humans (Grosholz 2005; Salo *et al.* 2007). Australia, like many other isolated land masses, has developed an ancient, unique and diverse complement of ecosystems due to its long isolation from other continents (Bowman 1998). However in recent times, the integrity of this unique environment is being compromised due to extensive habitat loss and degradation (Bradshaw 2012), and the introduction of many noxious species (Burney & Flannery 2005; Turney *et al.* 2008).

In invasion biology, the so-called 'genetic paradox' refers to the observation that many invasive species establish successfully despite often-reduced genetic variation arising from genetic bottlenecks imposed by small founder populations (Sakai *et al.* 2001). Such reduced variation can limit the species' capacity to adapt to its new environment and increase its risk of local extinction (Frankham & Ralls 1998). While many invasive species can escape the higher risk associated with inbreeding depression via asexual breeding or self-fertilization (Sakai *et al.* 2001), many others must solve the paradox via other mechanisms. High migration rates and repeat introductions are some ways in which low-diversity populations can be genetically rescued (Frankham 1997; Kolbe *et al.* 2004). Not surprisingly, propagule pressure (i.e. the number of individuals within, and frequency of, introductions) is a major determinant of invasion success (Brook 2004; Hayes & Barry 2008). This is because as propagule

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pressure increases, so too does the genetic diversity of the founding population(s), thus increasing the probability of adaptation to the novel environment (Crawford & Whitney 2010).

An important step in making efficient management decisions regarding invasive species is to determine the history of introduction and to quantify the rate of spread from introduction sites (Edwards *et al.* 2004; Hampton *et al.* 2004). Contemporary molecular techniques in conjunction with demographic and life history information are useful tools for understanding the dynamics, population structure, biology and colonization dynamics of plants and animals, including invasive species (Taylor *et al.* 2000; Sakai *et al.* 2001; Frankham *et al.* 2002; Ramsey *et al.* 2002; Spencer *et al.* 2005). This is because phylogeography, the approach we take here, provides the vital link between macro- and micro-evolutionary processes which allows us to trace genealogies across landscapes by quantifying the differences among populations (Lawson Handley *et al.* 2011). Linking genetic differences among populations with geographic information can help quantify how invading species use a landscape, identify how dispersal occurs, and gives insight into the mechanics of range expansion (Lawson Handley *et al.* 2011).

One of the more damaging and economically important introduced species in Australia is the Asian swamp buffalo (*Bubalus bubalis*; hereafter just 'buffalo') (Bradshaw *et al.* 2007a). Northern Australia is currently home to a widespread (Fig. 1) and growing population of at least 80 000 to 150 000 free-ranging buffalo (Bradshaw *et al.* 2007a). Buffalo are a major problem in Australia due mainly to the environmental damage they cause such as saltwater intrusion of wetlands and trampling of sensitive habitats (Braithwaite *et al.* 1984; Werner 2005; Werner *et al.* 2006; Petty *et al.* 2007), their potential threat to Australia's livestock industry as hosts for disease (Letts 1964; Standfast & Dyce 1972; Thomson 1977; Cousins & Roberts 2001; Ward *et al.* 2007; Bradshaw *et al.* 2012), and the danger they pose to human safety (Albrecht *et al.* 2009). Despite a few economic and traditional benefits the species provides (Albrecht *et al.* 2009; Collier *et al.* 2011), the largely negative ecological, economic and social impacts they incur argue for an urgent reduction of buffalo densities to limit damage to biodiversity, the economy and human welfare.

The first buffalo introduced to Australia (Fig. 1a) were a shipment of 16 animals onto Melville Island to the north of Darwin in 1826 (Letts 1962). Buffalo were first introduced to mainland Australia at the military settlement of Raffles Bay on the Cobourg Peninsula in 1827 from Kupang (now West Timor, Indonesia). Another 18 buffalo were obtained from Kisar Island (northeast of modern Timor-Leste) and introduced to nearby Victoria Settlement in Port Essington on the Cobourg Peninsula (Fig. 1a); thus, there is clear evidence of at least two separate introductions to the mainland (Letts 1962). Five years later in 1843, another 49 buffalo were introduced to Port Essington from Raffles Bay, thus facilitating interbreeding between the two initial introductions (Letts 1962). When Port Essington was abandoned in 1849, all breeding stock was released, and buffalo spread swiftly throughout the Northern Territory and formed vast herds across northern Australia (Fig. 1b). So swiftly did the population grow that by the 1880s there was a lucrative trade in wild-shot buffalo skins (between 1880 and 1911, an average of 4000 skins were collected annually) (Letts 1964; Tulloch 1969). Over the next 65 years, numbers and distribution increased (Fig. 1c) to an estimated 350 000 in the 1960s and 1970s and densities exceeded 20 buffalo km^{-2} in prime habitat (Bayliss & Yeomans 1989; Freeland & Boulton 1990). However, the total buffalo population experienced a severe reduction in abundance during the 1980s and 1990s in parts of its range (more than 90% reduction) under a national disease-extermination programme – the Brucellosis-Tuberculosis Eradication Campaign (Fig. 1d).

Despite this reduction, northern Australia remains a stronghold for a recovering buffalo population (Bradshaw *et al.* 2007a) (Fig. 1e). Quantifying differences in genetic structure for this population can provide an invaluable tool for quantifying subpopulation interactions and movement patterns. One of the main advantages of using genetic markers to assess population structure, diversity and mixing (movement) rates is that unlike traditional, expensive field-based methods (e.g. tracking or capture–mark–recapture), genetic studies do not require individual animals to be followed for long periods.

Recent molecular studies of buffalo have detailed the genetic diversity and population structure of buffalo populations within South-East Asia, Australia, Nepal and India (Barker *et al.* 1997b,c; Flamand *et al.* 2003; Vijh *et al.* 2008); however, a detailed and widespread analysis of genetic diversity and subpopulation structure is not available for the largest metapopulation of wild buffalo that now resides in Australia, and we have limited insight into how their rates of dispersal, carrying capacity and mating behaviour have changed since introduction. Here, we present a detailed analysis of the buffalo population from Australia using 10 microsatellites genotyped from 524 individuals from 11 geographically distinct subpopulations across the range of buffalo in northern Australia. We aim to (i) establish the rate and most probable history of spread from detailed microsatellite data derived from the 11 subpopulations and (ii) quantify the genetic distance and mixing rates between these subpopulations.

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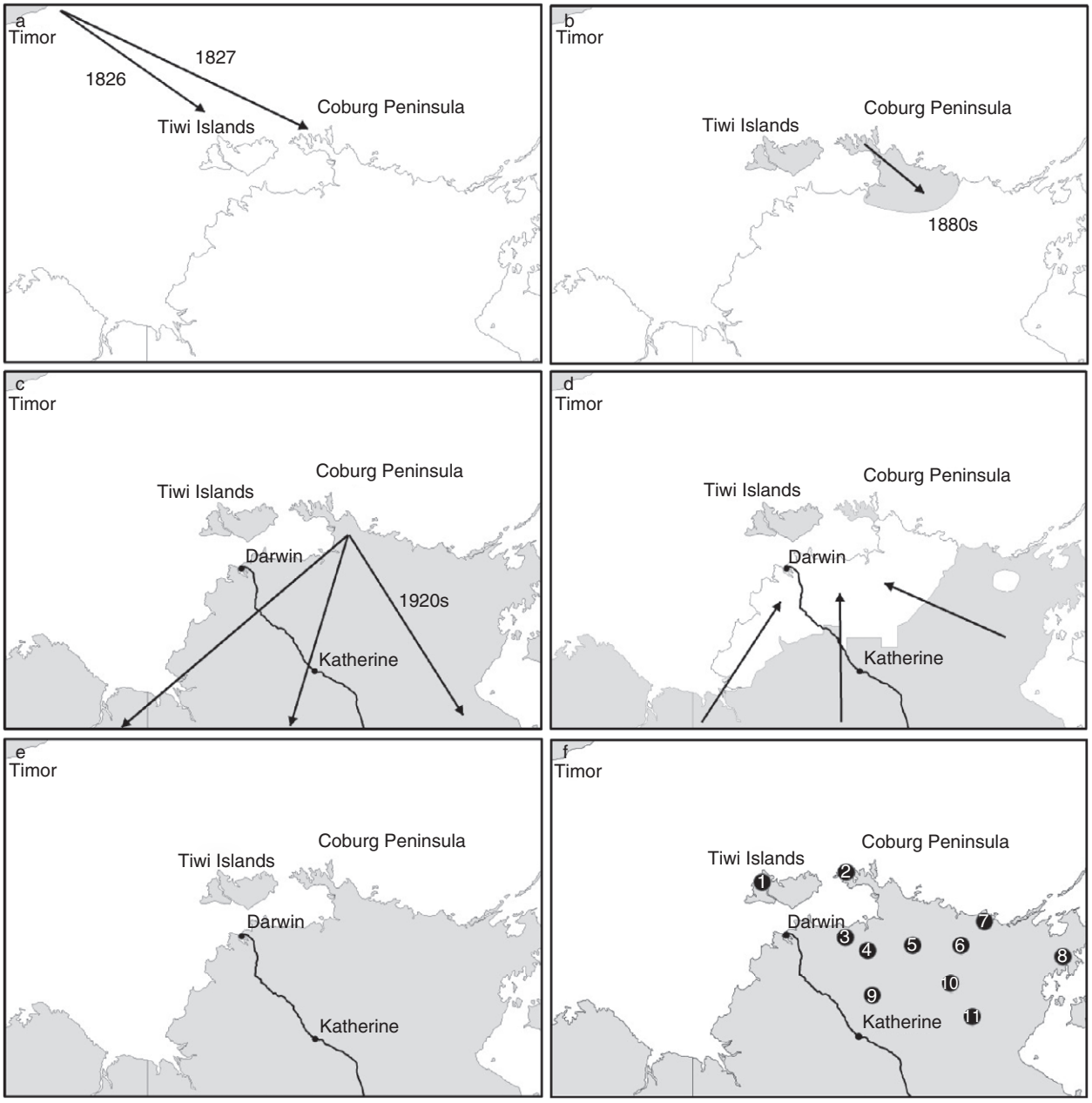

Fig. 1. Northern Australia showing (a) the two introductions (1826 and 1827) of swamp buffalo into Australia from Timor (b) the approximate range expansion (grey-shaded area) of buffalo after 50 years away from the Cobourg peninsula (c) the continued and wide distribution of buffalo across northern Australia in the 1920s (grey-shaded area) (d) the area (white area) from which buffalo were removed during the Brucellosis-Tuberculosis Eradication Campaign in the 1980s and 1990s (e) the current distribution (grey-shaded area) of buffalo across northern Australia showing a reinvasion of the areas from which they were removed during the Brucellosis-Tuberculosis Eradication Campaign and (f) the locations from where skin samples ($n = 524$) were collected across the Northern Territory including the founder populations on Melville Island (1) and the Cobourg Peninsula (2), the north-west corner of Kakadu National Park (KNP) (3), the buffalo farm within KNP (4), the greater Oenpelli region (5), our main study site centred at Kolorbidahdah (6), Ramingining (7), the greater Nhulunbuy region (8), the south-west corner of KNP (9), the Bulman region (10) and the Australian Wildlife Conservancy's Wongalara station (11).

METHODS

We collected 524 skin biopsies (6 mm diameter) from feral buffalo across the Northern Territory, Australia (Fig. 1f) representing 11 subpopulations. We stored each sample in 20% NaCl-saturated DMSO (dimethylsulfoxide) prior to DNA extraction. We extracted and purified DNA using standard SDS/proteinase K protocols and phenol/chloroform

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extractions (Sambrook & Russell 2001) and then amplified all extracted DNA. We applied polymerase chain reaction (PCR) amplifications following standard protocols using a Qiagen Multiplex PCR kit (Qiagen), to a final volume of 10 μL, which contained 5 ng of extracted DNA, 5 μL of 2 × Multiplex PCR Master Mix, and 0.2 μM of each multiplexed primer. We did PCR amplifications using a GeneAmp PCR System 2700 thermal cycler (Applied Biosystems) under the following conditions: initial denaturing at 95°C for 15 min; 25 cycles of denaturing at 94°C for 30 s, interspersed with annealing at 57°C for 1.5 min with each primer, extended for 1 min; and with a final extension at 72°C for 30 min. We measured the size of the PCR product using a 3100 Genetic Analyser and GENESCAN analysis software (Applied Biosystems, Foster City, CA, USA). We used 10 polymorphic microsatellite loci for genotyping (Barker *et al.* 1997a) and for quantifying relatedness between subpopulations. The 10 polymorphic loci were: CSSM008, CSSM019, CSSM022, CSSM029, CSSM032, CSSM038, CSSM041, CSSM043, CSSM047 and CSSM057. These loci were originally isolated from cattle and are known to be effective polymorphic DNA markers for use in buffalo (Moore *et al.* 1995).

Analysis

To quantify genetic variation we calculated the number of alleles *per* locus (A), observed heterozygosity (H_O) and expected heterozygosity (H_E). However, because of the variation in sample sizes, we assessed within-population genetic diversity using allelic richness scores (R_S) rather than measures comparing the absolute number of alleles in each subpopulation (El Mousadik & Petit 1996). We estimated allelic richness using program FSTAT 2.9.3.2 (Goudet 2002). To determine the probability that the alleles for any given gene are identical by descent, we calculated Wright's inbreeding coefficient (F_{IS}) for each of the 11 subpopulations and their probability of differing from zero based on 1000 permutations in FSTAT. To compare genetic diversity between buffalo in Australia and South-East Asia, we compared the genetic diversity indices from our samples with genotyped data at the same 10 microsatellite loci from a previous study of buffalo genetic diversity (Barker *et al.* 1997a).

We used analysis of molecular variance (AMOVA) to examine the hierarchical genetic structure among and within populations. We calculated overall and pairwise F_{ST} values and the probability that each pairwise F_{ST} value was not greater than zero based on 9999 permutations (in program GENALEX version 6.0) (Peakall & Smouse 2006). We examined genetic relationships among populations by constructing a phylogenetic tree: a neighbour-joining tree (Saitou & Nei 1987) using NEIGHBOR procedures incorporated within PHYLIP (Felsenstein 2004) and based on Nei's genetic distance (D_A) (Nei *et al.* 1983) within Microsatellite Analyzer 4.05 (Dieringer & Schlötterer 2003). We evaluated the robustness of the topology by 1000 bootstrap re-samplings using the SEQBOOT procedure in PHYLIP.

We investigated spatial genetic structure among populations using three approaches. First, we tested the isolation by distance (IBD) model for two datasets: (i) all 11 populations and (ii) only the 10 mainland populations (i.e. Melville Island population excluded). We examined the association between the matrix of the geographical distances and pairwise populations differentiation [$F_{ST}/(1 - F_{ST})$] (Rousset 1997) using a Mantel test with 9999 random permutations using GENALEX version 6.0 (Peakall & Smouse 2006). Second, we did a spatial analysis of molecular variance (SAMOVA) (Dupanloup *et al.* 2002) to determine clusters (groups) of populations that are geographically proximate and maximally differentiated from each other (Dupanloup *et al.* 2002). We applied the SAMOVA to the dataset containing all 11 populations and again for a dataset consisting only of the 10 mainland populations. Finally, we used STRUCTURE 2.3 (Hubisz *et al.* 2009) modified for populations where weak structure is anticipated, to infer inter-population structure and assign individuals to populations, subpopulations or clusters (K), and to estimate the assignment probability (q) of an individual buffalo belonging to a particular subpopulation K. STRUCTURE differentiates mixed populations on the basis of allele frequency at each locus using a Bayesian model-based clustering algorithm (Pritchard *et al.* 2000; Hubisz *et al.* 2009). This algorithm assigns individuals probabilistically to clusters to minimize Hardy–Weinberg disequilibrium and linkage disequilibrium. We performed 10^6 Markov Chain Monte Carlo (MCMC) iterations following a burn-in of 10^5 iterations using the model with admixture and correlated allele frequencies. Specifically, this procedure identifies the appropriate number of clusters using the statistic ΔK, which is based on the second-order rate of change in the log probability of the data between successive values of K (Evanno *et al.* 2005). We did 20 simulations for each K, and calculated ΔK for each cluster using the log probability derived from the above simulations (Evanno *et al.* 2005).

We estimated migration rates among the 11 subpopulations using BayesAss 1.3 (Staker 2006). This program applies a Bayesian method to multilocus genotypes and determines recent migration rates between populations over the last several generations by using MCMC simulations (Staker 2006). Importantly, this method can be applied to data where little population differentiation is apparent as with our data (overall $F_{ST} = 0.03$ for the 10 mainland populations). We used 3×10^6 MCMC iterations to quantify the migration rates between populations discarding the first 10^6 as burn-in iterations. Sampling occurred every 2000 iterations to determine the posterior probability distributions of the population allele frequencies and migrant proportions. We set delta, which defines the maximum amount a parameter can be changed each iteration, to the default value of 0.15.

RESULTS

Allelic diversity (A) for buffalo from the Northern Territory was depauperate when compared to that for buffalo in general (Barker *et al.* 1997a) (Table 1). However, because there is a strong relationship between the number of individuals sampled and the numbers of alleles observed, we determined rarefied allelic richness (allele diversity for each geographic area corrected for sample size); importantly, allelic richness (R_S) in Australia was low (2.35 to 3.00) when compared to buffalo from South-East Asia (2.68 to

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Table 1. Polymorphism of 10 microsatellite loci for *Bubalus bubalis*

Population		*n*	*A*	R_S	H_O	H_E	F_{IS}
Australia							
1	Melville I.	26	2.4	2.35	0.39	0.41	0.051
2	Cobourg	16	2.8	2.56	0.35	0.37	0.054
3	Kakadu	46	3.6	2.80	0.46	0.45	–0.016
4	Buffalo Farm	26	3.2	2.65	0.40	0.40	–0.005
5	South Kakadu	32	3.6	3.00	0.38	0.41	0.072
6	Oenpelli	29	3.2	2.62	0.39	0.37	–0.041
7	Kolorbidahdah	141	3.7	2.48	0.36	0.37	0.014
8	Bulman	116	3.9	2.60	0.40	0.40	0.002
9	Wongalara	70	4.0	2.75	0.41	0.41	0.022
10	Ramingining	10	2.5	2.50	0.46	0.40	–0.161
11	Nhulunbuy	11	2.6	2.56	0.43	0.40	–0.085
Thailand							
12	Surin	25	6.3	5.26	0.67	0.70	0.048
Malaysia							
13	Trengganu	25	5.2	4.61	0.63	0.67	0.066
14	Sabah	25	3.2	2.93	0.52	0.47	–0.112
15	Sarawak	25	2.8	2.68	0.53	0.50	–0.061
Indonesia							
16	Bogor	25	4.8	4.06	0.63	0.61	–0.037
17	Sulawesi	25	4.4	3.93	0.59	0.61	0.039
Philippines							
18	Musuan	26	5.9	4.50	0.60	0.62	0.032

Shown are number of individuals genotyped (524) for each locus, number of alleles per locus (*A*), allelic richness (R_S), observed heterozygosity (H_O), expected heterozygosity at Hardy–Weinberg equilibrium (H_E) and Wright's inbreeding coefficient (F_{IS}). Sources: Populations 1–11 (this study); Populations 12–18 (Barker *et al.* 1997a).

5.26) (Table 1). Both observed and expected heterozygosity in Australia ($H_O = 0.35$ to 0.46; $H_E = 0.35$ to 0.45) were also lower than in the South-East Asian meta-population ($H_O = 0.52$ to 0.67; $H_E = 0.50$ to 0.70). Such low diversity can be attributed either to inbreeding or to a small founding population, but we found evidence that Australian buffalo are mating randomly (i.e. no evidence Wright's inbreeding coefficient $F_{IS} > 0$ in any population). Low genetic diversity is expected from a population that had a small founding population (fewer than 80 buffalo) (Barker *et al.* 1997b; Bradshaw *et al.* 2007b).

Melville Island buffalo differed markedly from the 10 mainland subpopulations based on three different methods: neighbour-joining tree, IBD and STRUCTURE, thus confirming the historical records that there were separate introductions of buffalo into Australia onto Melville Island and then from a separate founder population to the Cobourg Peninsula (Fig. 1). While we found some support ($R^2 = 0.29$, $P < 0.01$) for IBD when all 11 populations were included in the analyses (Fig. 2a), there was no evidence for IBD when comparing only the mainland populations ($R^2 = 0.07$, $P = 0.18$; Fig. 2b), indicating that the mainland population is panmictic. Moreover, the unrooted neighbour-joining tree (Fig. 3) and STRUCTURE analysis (Appendix S1, online supplementary material) corroborated the finding for little genetic differentiation

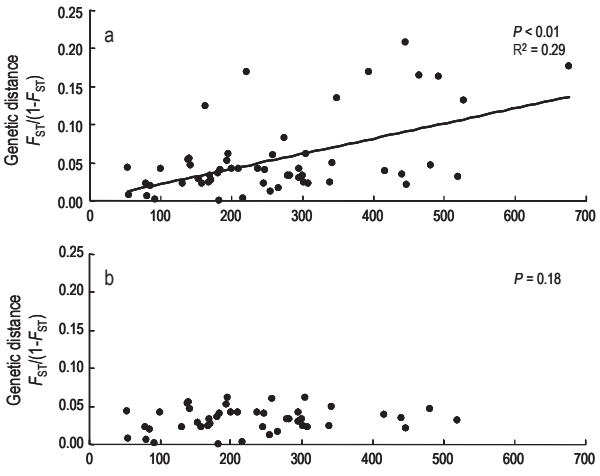

Fig. 2. Relationships between geographical distance (km) and pairwise genetic distance for (a) all 11 Australian populations and (b) only the 10 mainland populations in the mainland.

and structure in the mainland populations, but they nonetheless revealed that the Kolorbidahdah (6), Ramingining (7) and the greater Nhulunbuy region (8) subpopulations formed an eastern cluster. The Cobourg Peninsula (2), the north-west corner of Kakadu National Park (KNP) (3), the buffalo farm

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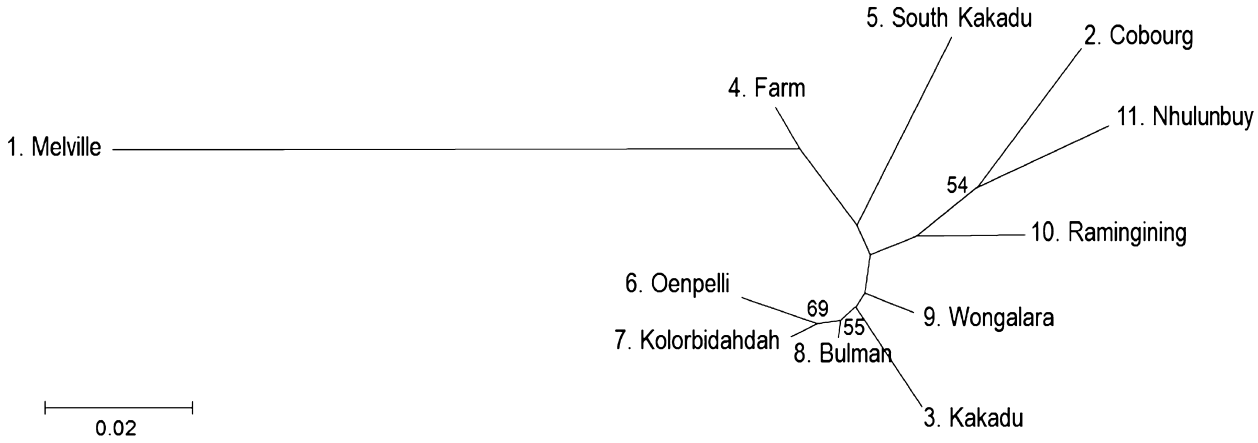

Fig. 3. An unrooted neighbour-joining tree of Nei's genetic distance (*DA*).The values on the tree indicate the support, in percentages, of supported topologies from 1000 bootstrap replicates.

Table 2. Summary of the AMOVA results

Source of variance	d.f.	SS	MS	$\hat{\sigma}^2$	%	
All populations						
Among	10	95.2	9.52	0.09	4%	$F_{ST} = 0.04$ $P = 0.001$
Within	1035	2048.5	1.98	1.98	96%	
Mainland populations (excl. Melville Island)						
Among	9	63.1	7.01	0.06	3%	$F_{ST} = 0.03$ $P = 0.001$
Within	984	1944.3	1.98	1.98	97%	

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), estimated variance of components ($\hat{\sigma}^2$), per cent of total variance (%) and test probability (*P*).

within KNP (4), the greater Oenpelli region (5), the south-west corner of KNP (9), the Bulman region (10) and the Australian Wildlife Conservancy's Wongalara station (11) clustered together in a second central-western mainland grouping (Appendix S1, online supplementary material).

Most of the observed variability (96.0% of total AMOVA variance) occurred within subpopulations and little variation (4.0% of the total AMOVA variance) was evident between subpopulations (Table 2). The result was similar for just the 10 mainland subpopulations, such that 97% of the total variance was observed within subpopulations. Nonetheless, while the mainland subpopulations were relatively well mixed, there is some evidence for between-subpopulation structure as revealed by the pairwise F_{ST} comparisons across all 11 subpopulations (Table 3). The overall F_{ST} estimate for the 11-way comparison was 0.05 ($n = 11$; $P = 0.001$), indicating moderate gene flow between subpopulations. However, the subpopulation pairwise F_{ST} values were varied, ranging from 0.001 to 0.172; this demonstrated that most subpopulations did not differ from each other (Table 3). We observed the most profound difference between subpopulations (Table 3) between Melville Island and the mainland subpopulations ($F_{ST} = 0.076$ to 0.172). There was considerable gene flow between Oenpelli and its two immediate-neighbour subpopulations: Kolorbidahdah and Bulman (Appendix S1, online supplementary material). In contrast, the evidence for some restricted gene flow between the geographically closer neighbouring Bulman and Kolorbidahdah subpopulations ($F_{ST} = 0.006$, $P = 0.007$) suggests that distance itself is not a good predictor of isolation (Fig. 2). In the latter case, the sandstone outcrops between these subpopulations might restrict movement (McMahon *et al.* 2010).

Assignment test results revealed that many animals originate from distant subpopulations (Appendix S1, online supplementary material) rather than adjacent subpopulations, corroborating the findings of the IBD analysis. We found that most of individuals from Melville Island were assigned to a single cluster – Cluster 1, while Cluster 2 membership coefficients at $K = 3$ and 4 were high for the individuals of Kolor-

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bidahdah, Oenpelli and Bulman. The Cluster 3 membership coefficients at $K = 3$ and 4 were high for the individuals of Kakadu, South Kakadu, Farm, Wongalara, Ramingining and Nhulunbuy, and the *F*-values of the Cluster 3 at $K = 3$ and 4 were relatively low.

Migration rates among most localities were low such that each subpopulation was characterized by a high proportion (more than 67%) of local individuals to migrant individuals (less than 30%) (Fig. 4), suggesting subpopulations were generally isolated. However, migration did occur and was most common from Arnhem which acted as a source subpopulation for the Bulman, Oenepelli and Wongalara subpopulations, so that Arnhem migrants accounted for 30.1%, 25.7% and 18.3% of these three subpopulations, respectively. There was also migration into Bulman from neighbouring Arnhem (30% of the Bulman subpopulation probably originated from Arnhem approx. 60 km away).

DISCUSSION

Genetic variation in Australian buffalo is low compared to that found in buffalo from India and South-East Asia (Barker *et al.* 1997a; Vijh *et al.* 2008), as expected from a population derived from a small founder group. Despite this reduced genetic variation and the small size of the founder population ($n < 100$), the Australian meta-population has thrived (Tulloch 1969; Tulloch 1970; Freeland & Boulton 1990) and spread outwards from their introduction site on the Cobourg Peninsula at high rates over the last 160 years (invading approx. 224 000 km^2 over that time, or an average of 1400 km^2 year^{-1}). The lack of evidence supporting any phylogeographic structure in the north Australian population of buffalo is likely due in part to the complicated and uncertain introduction histories and the seemingly high rates of interchange within the broader mainland population.

Contemporary buffalo subpopulations nonetheless express some regional differentiation, which probably reflects their propensity to breed mainly with their immediate neighbours once densities have reached local carrying capacity, and dispersal-limiting geographic hurdles such as mountain ranges and seasonally impassable rivers. Subpopulations can also develop when: (i) a larger population is reduced to a few individuals that then interbreed and form a new subpopulation that exhibits the genetic traits of the founders; (ii) a small number of migrants with a distinctive genetic composition is introduced into a novel environment after local culls; or (iii) separate new introductions occur from novel sources. While it is difficult to explain definitively the current limited population structure, we propose that it is a consequence of buffalo moving swiftly across the landscape invading their new, productive and predator-free habitats; but once established, they tend to be bound to relatively limited areas (Tulloch 1970, 1978) and rarely move long distances unless otherwise compelled to by high intra-specific resource competition (Carrick *et al.* 1990).

Buffalo are an acknowledged ecological pest in Australia, but perhaps more importantly, they pose a

Table 3. Pairwise F_{ST} values and Type I error probabilities (above diagonal) among 11 populations of *Bubalus bubalis*

	Melville	Cobourg	Kakadu	Farm	S Kakadu	Oenpelli	Kolorbidahdah	Bulman	Wongalara	Ramingining	Nhulunbuy
Melville		0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Cobourg	0.110		0.001	0.009	0.006	0.003	0.001	0.001	0.002	**0.100**	0.050
Kakadu	0.144	0.051		0.001	0.001	0.001	0.001	0.001	0.001	**0.054**	0.003
Buffalo Farm	0.076	0.035	0.041		0.001	0.001	0.001	0.001	0.033	**0.119**	0.029
South Kakadu	0.119	0.032	0.044	0.041		0.007	0.001	0.001	0.001	0.029	**0.055**
Oenpelli	0.145	0.056	0.038	0.053	0.024		**0.068**	**0.301**	0.003	**0.082**	0.029
Kolorbidahdah	0.172	0.057	0.040	0.058	0.041	0.007		0.007	0.001	**0.069**	0.005
Bulman	0.141	0.047	0.023	0.050	0.031	0.001	0.006		0.001	**0.049**	0.014
Wongalara	0.116	0.037	0.021	0.012	0.040	0.027	0.028	0.021		**0.322**	0.009
Ramingining	0.140	0.023	0.023	0.017	0.029	0.022	0.019	0.022	0.003		**0.418**
Nhulunbuy	0.150	0.031	0.045	0.034	0.021	0.031	0.038	0.032	0.041	0.000	

Probabilities in bold indicative of lack of strong evidence for pairwise differences.

Fig. 4. Northern Australia and the locations of the 11 populations of Asian swamp buffalo sampled showing the immigrant proportion and the direction of movement of each population depicted with either a broken line when migration into the population is less than 10% or a solid line when it is more than 10%. Each panel represents one of the 11 populations so that: (a) depicts migration into Melville Island – population 1, (b) the Cobourg Peninsula – population 2, (c) the north-west corner of Kakadu National Park (KNP) – population 3, (d) the buffalo farm within KNP – population 4, (e) the greater Oenpelli region – population 5, (f) our main study site centred at Kolorbidahdah – population 6, (g) Ramingining – population 7, (h) the greater Nhulunbuy region – population 8, (i) the south-west corner of KNP – population 9, (j) the Bulman region – population 10, and (k) the Australian Wildlife Conservancy's Wongalara station – population 11.

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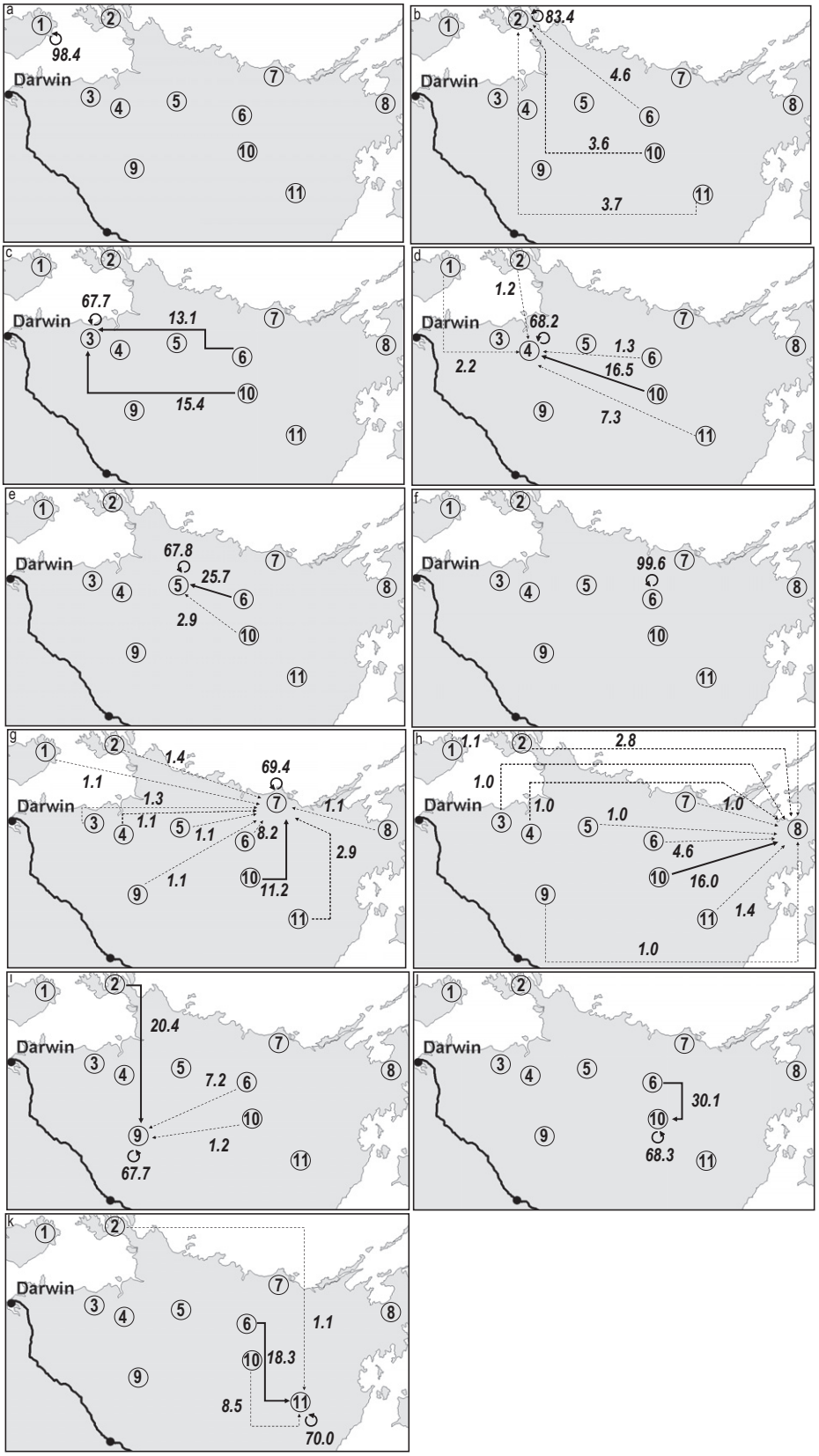

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serious disease threat as hosts for economically crippling diseases such as tuberculosis (Bradshaw *et al.* 2012) or foot-and-mouth. If these livestock diseases are introduced into Australia, they would severely encumber the country's trade in livestock which is currently dependent on its disease-free status. Consequently, understanding how buffalo use their environment (movement and dispersal patterns) and how susceptible they are to disease represent key components of managing potential disease incursions. We found evidence for limited population structure within the Australian buffalo population, suggesting that animals are mixing freely across their distribution. The uninhibited contact between individuals across their range suggests that any introduced virulent pathogen could be easily and swiftly spread across the entire population.

That the buffalo population has low genetic diversity might also pose an enhanced epidemiological threat given that estimators of genetic diversity, such as mean heterozygosity, have been correlated with fitness traits, such as survival, disease susceptibility and reproductive success (O'Brien & Evermann 1988; McCallum 2008). Currently there is no information linking genetic diversity and disease susceptibility in buffalo in Australia, but given the low genetic diversity we observed, the population's widespread and well-mixed distribution interspersed among the domestic cattle population (Bradshaw *et al.* 2007a; McMahon & Bradshaw 2008), it follows that there is some urgency to investigating the disease susceptibility–heterozygosity relationship in light of its potential implications for the Australian economy.

While defining the success of invading species based on their ecological traits is useful, such explanations are limited without some understanding of the introduction history and 'propagule pressure' (Pollock & Montague 1991). Propagule pressure can be defined as a composite measure of the number of individuals of a non-native species (propagule size) introduced to a novel environment and the number of introduction events (propagule number) into the new host environment (Lockwood *et al.* 2005). An important component of propagule pressure is the amount of genetic variation contained within the founder population; more individuals are likely to host greater genetic diversity, thus reducing extinction risk from inbreeding depression, and improving the chances of successful adaptation to and exploitation of the novel environment (Crawford & Whitney 2010). Another introduced large ungulate, the banteng (*Bos javanicus*) has demonstrated comparable success in northern Australia (Bradshaw *et al.* 2006), despite an even lower heterozygosity (Bradshaw *et al.* 2007b) than buffalo. Despite these small founder populations and limited genetic variation, the success (defined here as population persistence) of buffalo and banteng demonstrates how even inbred bovids can overcome the potential genetic barriers posed by inbreeding depression to successful invasion and spread (and associated high environmental impacts) if released from high predation, parasitism and disease rates typical of native habitats (Freeland 1990).

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Membership of each buffalo in each genetic cluster inferred by the STRUCTURE analysis.

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