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New insights on the history of canids in Oceania based on mitochondrial and nuclear data

Kylie M. Cairns[1] · **Alan N. Wilton**[1]

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Abstract How and when dingoes arrived in Oceania poses a fascinating question for scientists with interest in the historical movements of humans and dogs. The dingo holds a unique position as top terrestrial predator of Australia and exists in a wild state. In the first geographical survey of genetic diversity in the dingo using whole mitochondrial genomes, we analysed 16,428 bp in 25 individuals from five separate populations. We also investigated 13 nuclear loci to compare with the mitochondrial population history patterns. Phylogenetic analyses based upon mitochondrial DNA and nuclear DNA support the hypothesis that there are at least two distinct populations of dingo, one of which occurs in the northwest and the other in the southeast of the continent. Conservative molecular dating based upon mitochondrial DNA suggest that the lineages split approximately 8300 years before present, likely outside Australia but within Oceania. The close relationship between dingoes and New Guinea Singing Dogs suggests that plausibly dingoes spread into Australia via the land bridge between Papua New Guinea and Australia although seafaring introductions cannot be rejected. The geographical distribution of these divergent lineages suggests there were multiple independent dingo immigrations. Importantly, the observation of multiple dingo populations suggests the need for revision of existing conservation and management programs that treat dingoes as a single homogeneous population.

Keywords Australia · Dingo · Divergence estimates · Mitochondrial DNA · Neolithic · Nuclear DNA · Phylogeography · Population genetics

Introduction

Large carnivores, such as the dingo, are fundamental to the resilience of biodiversity and ecosystem functioning, yet face extinction due to extensive lethal control programs by humans (Ripple et al. 2014). The dingo is listed as vulnerable (decreasing) on the IUCN Red List, only extending across 84 % of its historical range and threatened by hybridisation with European domestic dogs (Ripple et al. 2014). In other large carnivores genetic subdivision has been linked to differences in habitat dispersal preference (Randall et al. 2010; Sacks et al. 2004), prey specialisation (Carmichael et al. 2001) and environmental conditions (Stenseth et al. 2004). Despite this, there is a lack of scientific research concerning population size, genetic integrity and biogeography of dingoes. Subsequently ongoing management and control programs are not based on rigorous scientific data. Understanding the origin, history and modern population structure of large carnivores is important for establishing adequate conservation strategies. A goal of this study is to investigate hypotheses concerning the origin, history and population structure of the Australian native dog, the dingo.

In Australia there is extensive historical and current conflict between livestock holders and dingoes. Dingoes are managed and culled throughout Australia, specifically in "prime" sheep country such as rural Queensland, New

Alan N. Wilton: Deceased.

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✉ Kylie M. Cairns
kylie@kyliecairns.com

[1] School of Biotechnology and Biomolecular Sciences, University of New South Wales, Sydney 2052, Australia

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South Wales and Victoria (Fleming et al. 2001). Legislatively, the dingo is considered to be both a native animal requiring protection and a controlled agricultural pest (Davis 2001; Fleming et al. 2001). This inconsistency is largely due to lack of recognition of the dingoes' ecological importance both as an apex predator and in controlling invasive mesopredators such as foxes and cats (Carthey and Banks 2012; Letnic et al. 2010, 2012; Moseby et al. 2012; Wallach et al. 2010). The reining dogma that dingoes arrived with humans as part of the Neolithic also impacts the public's perception negatively (Savolainen et al. 2004).

It is often posited that dingoes were brought to Australia as a result of human movements (Corbett 1995; Macintosh 1975; Savolainen et al. 2004). There are two major pre-European human movements that occurred in the Australasian region: (1) Indigenous Australians settled in Australia between 46,000 and 60,000 years before present (BP) (Gibbons 2001; McEvoy et al. 2010; Milham and Thompson 1976; van Holst Pellekaan 2013), and (2) the Neolithic expansion occurred approximately 5500 years BP (Bocquet-Appel 2011; Diamond 2002). Australia and Papua New Guinea were once joined by a land bridge forming the continent of Sahul. Approximately 6500–8500 years BP the land bridge between Papua New Guinea and Australia flooded forming two geographically distinct islands. The Neolithic expansion was responsible for replacing many hunter-gatherer cultures and introducing agriculture and domesticated animals, including pigs and chickens, to South East Asia and Oceania but not Australia (Bocquet-Appel 2011; Diamond 2002; Gibbons 2001; Haak et al. 2010; Larson et al. 2007, 2010). However, a range of evidence suggests that dingoes are unlikely to have been brought to Australia by post-agricultural people (Neolithic), as commonly posited, but rather with a hunter-gather culture (Arendt et al. 2016; Fillios and Taçon 2016; Freedman et al. 2014).

Ongoing controversy surrounds the origin of dogs. Most hypotheses suggest that dogs in South East Asia and Polynesia migrated commensally with humans but the timing of this event is debated (Frantz et al. 2016; Pang et al. 2009; Sacks et al. 2013; Shannon et al. 2015; von Holdt et al. 2010; Wang et al. 2013, 2016). One hypothesis based on nuclear single nucleotide polymorphism (SNP) data suggests that the primary site of canine domestication was in the Middle East, with only a few ancient dogs, such as the dingo, sharing affinity with Asian wolves (von Holdt et al. 2010). A Y chromosome based hypothesis suggests that a Neolithic expansion of dogs from Southeast Asia partially replaced older dogs in the west and north (Sacks et al. 2013). An alternate hypothesis based on mitochondrial DNA (mtDNA) data proposes the primary dog domestication site as Southern China about 16,000 years BP (Pang et al. 2009). However, complete mtDNA genomes of ancient canids from Eurasia suggest that all modern dogs are phylogenetically related to ancient or modern canids from Europe and molecular dating suggests domestication of dogs began 18,800–32,100 years BP (Thalmann et al. 2013). Whole genome sequencing data suggests that wolves and dogs diverged in excess of 27,000 years BP (Skoglund et al. 2015; Wang et al. 2013, 2016). Whole genome sequencing studies present multiple sites of domestication, South East Asia (Wang et al. 2013, 2016) or Central Asia (Shannon et al. 2015). Another hypothesis by Frantz et al. (2016) suggests that dogs may have been domesticated from two separate wolf populations, one in Asia and one in Eurasia. Certainly, the timing, evolution and movement of dogs around the globe is a complex matter that is yet to be solved and dingoes represent an important piece of this puzzle.

The oldest fossilised dingo remains, excavated in southern Australia, have been dated to 3500 years BP, suggesting an arrival date for dingoes of 5000 years BP during the Neolithic expansion (Gollan 1984; Macintosh 1975). Fossil evidence provides a solid minimum divergence estimate, if the fossil can be accurately identified; however, the fossil record is patchy and so divergence estimates may be underestimates. This is important in Island South East Asia and northern Australia as acidic soil and tropical conditions may have inhibited fossil recruitment and persistence (Kidwell and Flessa 1996; Tappen 1994).

Molecular data has previously been used to unravel geographic subdivision and estimate the time to the most recent common ancestor (MRCA) in a variety of animals including dingoes. Dating divergence times typically rely upon estimating a substitution rate using sequence data along with biogeographic, pedigree, geological or archaeological evidence. The initial genetic study on dingoes using the mtDNA control region estimated the time to the MRCA at approximately 5000 years BP (Savolainen et al. 2004). A more extensive study of dingoes and South East Asian island dogs re-estimated the divergence of the mtDNA to 4600-18,300 years BP using the same control region (Oskarsson et al. 2011). A Y chromosome study on 47 wild and captive dingoes largely from eastern Australia identified a pattern of West versus East biased allele distribution and suggests that dingoes may have immigrated twice (Ardalan et al. 2012). A close relationship to the NGSD was also inferred from Y chromosome data (Ardalan et al. 2012; Sacks et al. 2013). However uneven geographic sampling in these studies made it difficult to elucidate geographical and population level patterns of genetic variation.

The presence of genetic subdivision within the dingo has been postulated for decades but has yet to be empirically investigated (Corbett 1995; Savolainen et al. 2004). We assess the genetic relationships between five geographically distinct dingo populations, the NGSD and two closely

related dogs from South East Asia. These five dingo populations enable us to investigate the genetic variation spanning the Australian continent. NGSD and Indonesian dogs are included as plausible relatives of dingoes. All dingo samples were sourced from wild populations. We investigate genetic patterns of phylogeny and differentiation using whole mtDNA genome data and 13 nuclear loci from three functional groups: the major histocompatibility complex (DLA) genes, olfactory receptor genes (cfOR) and coat colour genes (CC). We then cautiously employ the whole mtDNA genome data and the published canid mtDNA substitution rate to the estimate the divergence times of lineages. Based upon these data we hypothesise that there are at least two dingo populations and that plausibly dingoes immigrated into Australia more than once.

Materials and methods

Canid sampling

Mitochondrial DNA

We sampled five dingoes each from each of five geographically distinct regions: the Kimberley in northwestern Australia, the Gibson and Simpson Deserts in western and central Australia respectively, Fraser Island in eastern Australia and the Australian Alpine region in southeastern Australia (Table S1, Fig. S1). Tissue and/or blood was collected and pre-screened for domestic dog introgression, using a microsatellite based test developed by ANW (Wilton 2001). These microsatellites are used to distinguish between domestic dogs and dingoes. Only dingoes that were assessed as pure by this method were assigned to this project (Stephens 2011; Wilton 2001).

Given the inferred close relationship of the dingo to the wild dog of Papua New Guinea we include a NGSD sample (Ardalan et al. 2012; Sacks et al. 2013). A single NGSD was used in the whole mtDNA dataset as captive NGSD predominately derive from the same maternal lineage. NGSD samples were collected from captive animals in the USA (Table S1). NGSD samples were assessed as pure by pedigree and microsatellite assessment. We also included dog samples from the Indonesian islands of Bali and Kalimantan as outgroups (Oskarsson et al. 2011; Savolainen et al. 2004). The Bali dog sample was collected from Bali, Indonesia and the Kalimantan dog sample was collected from Mallinau, Kalimantan, Indonesia (Table S1).

Nuclear DNA

The same dingo sampling design was followed for nuclear DNA investigations. However, due to difficulties amplifying nuclear markers the Fraser 1 and Simpson 4 individuals were replaced with samples from the same geographical region (Fraser 6 and Simpson 6). Further, we expected additional nuclear variation from the NGSD, so we increased the number of samples to five. Finally, we had difficulty amplifying the nuclear markers from the Bali dog sample and it was excluded from all nuclear studies.

Genetic investigations

To capture all the variation in the mtDNA we sequenced the complete mitochondrial genome. Thirteen nuclear regions were identified as plausibly containing genetic variation in dingoes (Anderson et al. 2009; Kennedy et al. 2007; Robin et al. 2009; Tacher et al. 2005). This included genes involved in immunity (DLA), olfactory reception (cfOR) and coat color (CC).

DNA was extracted using a Qiagen DNeasy kit (Qiagen Sciences, Germantown, USA) and the loci of interest were amplified from 25 dingoes, 1 NGSD and 2 Indonesian dogs using PCR (Table S2 and Table S3). PCR amplicons underwent ExoSAP-IT® (USB Amersham, Buckinghamshire, UK) purification prior to sequencing. Sanger sequencing was performed on the purified templates using BigDye terminator v3.1 (Applied Biosystems Inc., Foster City, USA).

Sequence chromatograms were visualised and manually edited to remove erroneous base calls, such as unincorporated dyes, using SEQUENCHER 5.1 (Gene Codes corp., Ann Arbor, USA). Mutations observed in a single individual were corroborated by re-amplifying and sequencing the region of interest and comparing the independent chromatograms. No instances of mismatch, damage or misincorporation were identified. MtDNA genome contigs were then constructed for each individual.

Heterozygous autosomal sequences were resolved in a two-step approach. First, we used the Phase v2.1 algorithm implemented in DNASP v5.10.1 (Librado and Rozas 2009) to identify individuals carrying unique alleles. Second each unique allele was cloned to fully resolve the sequence. Cloning was performed using a CloneJet kit according to the manufacturers instructions (Thermo Fisher Scientific Inc., Waltham, USA).

Phylogeny and clustering analyses

Whole mtDNA phylogenetic analysis

According to the literature, the most suitable phylogenetically distinct outgroup taxa for a critical examination of dingoes are South East Asian dogs, most specifically those carrying the A29 control region haplotype (Oskarsson et al. 2011; Savolainen et al. 2004). Therefore we include A29_10100 (GenBank # EU789776), from Guizhou in

southern China and two Indonesian dogs from the islands of the Bali and Kalimantan as outgroups.

JMODELTEST 2.3.1 (Darriba et al. 2012) was run on the dataset of 25 dingoes, NGSD, Bali, Kalimantan and A29_10100 dogs to evaluate the most suitable substitution model for the dataset. A GTR + G + I substitution model was chosen using the Akaike selection criteria, and was used in subsequent mtDNA phylogenetic analyses. Constant population size coalescent models are generally most suitable for intraspecific datasets (Kingman 1982). Therefore Bayesian phylogenetic analyses were performed in BEAST 1.7.4 (Drummond et al. 2012) with a constant population size coalescent model.

A strict clock is suitable for analyses that incorporate intraspecific data (Brown and Yang 2011), therefore mtDNA phylogenetic analyses were run with a strict molecular clock (normally distributed with mean = 1.28×10^{-8} mutations^{-1} site^{-1} year^{-1} and SD = 3.27×10^{-9}) (Pang et al. 2009). All runs were optimised for Markov chain Monte Carlo (MCMC) chain steps to ensure the estimated sampling size was above 200 in TRACER 1.5 (Rambaut and Drummond 2007). Sampling occurred every 5000 steps with a 10 % burn-in.

Nuclear Bayesian clustering analysis

To further investigate population subdivision within the dingo we employed a Bayesian population clustering analysis as implemented in STRUCTURE (Hubisz et al. 2009). Briefly, each individual's nuclear identity was summarised by coding diploid haplotypes at each of the thirteen nuclear loci. STRUCTURE was run with 100,000 MCMC chains, a 10 % burn-in and the default Admixture model settings using sampling location information. Scenarios for K = 1 to K = 10 were run, with 10 iterations per K value. The best K was chosen by the Evanno method (Evanno et al. 2005). CLUMPP v1.1.2 (Jakobsson and Rosenberg 2007) was used to compare the Q matrices of the ten runs for the best K and an average Q-plot was constructed using DISTRUCT v1.1 (Rosenberg 2004). It is important to note that for the STRUCTURE analysis only dingo and NGSD samples were included. STRUCTURE analyses were repeated excluding 2 loci (cfOR0007 and cfOR0034) observed to be under selection. Simple Chi squared contingency table analyses were performed to investigate whether the observed allele frequency pattern at the cfOR0007 and cfOR0034 cfOR gene regions departed from a random pattern indicating geographic subdivision.

Statistical analyses

MtDNA

Measures of genetic differentiation (F_{ST}) between geographical populations (Alpine, Fraser, Simpson, Gibson, Kimberley, Papua New Guinea and Indonesia) were calculated in ARLEQUIN 3.5 (Excoffier and Lischer 2010). F_{ST} calculations are frequently used in population genetics to investigate whether populations of the same species are subdivided or still experiencing high levels of gene flow.

To investigate if the sampled mtDNA sequences are evolving in a manner consistent with a strictly neutral equilibrium pattern of evolution Tajima's *D* and Fu and Li's *F** tests were performed using DNASP v5.10.1. Neutrality tests can compound the effects of demography and selection, therefore selection can really only be reliably identified when the population is at equilibrium (Fu 1997; Tajima 1989). If a departure from demographic equilibrium is observed then significant values should be interpreted with caution. To avoid violating the assumptions of the Neutrality tests, such as the absence of population subdivision, statistics were calculated on phylogenetic lineages separately.

Nuclear

Statistical analyses were calculated for each autosomal locus using the fully resolved datasets in DNASP v5.10.1. F_{ST} analyses were implemented in ARLEQUIN 3.5. Tajima's *D* and Fu and Li's *F** tests were performed using DNASP v5.10.1. Neutrality statistics were calculated for genetic lineages, as defined by the mitochondrial phylogeny, separately.

Estimating divergence time and substitution rate of mtDNA

We estimated divergence times using 28 whole mtDNA genomes and the published estimate of the whole mtDNA substitution rate of canids, which was calibrated using the coyote-wolf fossil divergence time of 1.5–4.5 million years BP (Pang et al. 2009). This is 1.28×10^{-8} mutations-1 site-1 year-1 with a standard deviation of 3.27×10^{-9} (Pang et al. 2009). We calculated molecular divergence by implementing a tMRCA statistic for each divergence event observed, running the analysis in BEAST 1.7.4 and visualizing the results in TRACER 1.5. A potential problem with these approaches is that they may overestimate the intraspecific divergence time because slightly deleterious mutations can accumulate within species or populations but not go to fixation between them (Ho et al. 2011; Ho and Larson 2006).

A complimentary analysis was carried out to investigate the divergence estimates of dingoes using a tip-calibrated substitution rate (Thalmann et al. 2013). The Thalmann et al. (2013) dataset was analysed using BEAST 1.8.0 using tip-date calibrations (as per Thalmann et al., 2013) and a GTR + G substitution model. The estimated substitution rate was 7.7027×10^{-8} mutations^{-1} site^{-1} year^{-1} with a

standard deviation of 5.4848×10^{-9}. Our 28 mtDNA genome dataset was analysed in BEAST 1.8.0 with this new substitution rate and divergence estimates were visualised in TRACER 1.6, as above.

Results

Genetic analyses

The dingo, NGSD, Bali and Kalimantan dog mitochondrial genome architecture is identical to that of other dogs. Excluding the repeat structure located within the control region that is difficult to unambiguously align, the mtDNA genome analysis included 16,428 bp.

Among the 25 dingo mtDNA genomes sequenced, 20 haplotypes were detected (Table S1, JX0088671-JX088693). The NGSD (JX088674), Bali (JX088690) and Kalimantan (JX088689) dogs each carried distinct mitochondrial haplotypes. In the 20 dingo and one NGSD haplotypes observed there were 78 segregating sites (excluding indels) and 17 non-synonymous polymorphisms. A single mitochondrial haplotype was detected in all of the dingoes from Fraser Island. This is consistent with a small maternal foundation population.

Coding regions of 13 nuclear loci were sequenced. There were a total of 87 segregating sites from three DLA genes, 127 from seven cfOR genes and 14 from three CC genes (Table S1, KF586867-KF587269).

Phylogeny and clustering analyses

Whole mtDNA phylogenetic analysis

Bayesian analyses run on the 28 genomes sequenced and the Chinese A29_10100 dog show that the Bali, Kalimantan and A29_10100 dogs sit outside the monophyletic NGSD and dingo lineage (Fig. 1).

A notable result of this phylogenetic analysis is the strong biogeographic clustering of two lineages, with a southeastern (SE) population and predominantly northwestern (NW) population. Due to the low posterior probability support of 0.4 it is not clear, however, whether the NGSD is distinct from the SE lineage of dingoes. The single exception to the biogeographic clustering is that one dingo (Alpine 5) collected from the Australian Alpine region in the southeast of Australia was found to carry a NW mtDNA lineage haplotype (Fig. 1).

Nuclear Bayesian clustering analysis

Using the Evanno et al. (2005) method, the most optimal population clustering model was K = 3 (Fig. 2), however

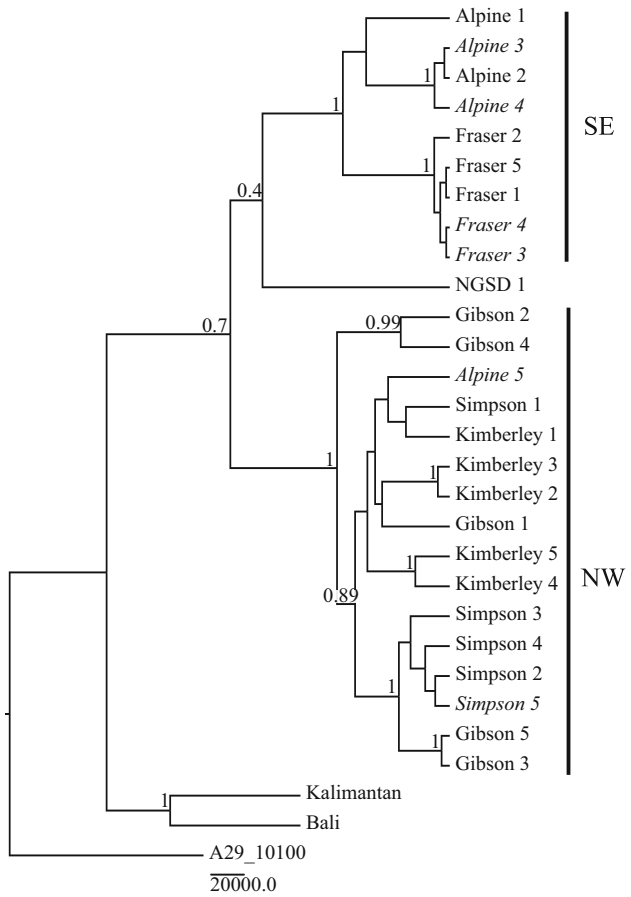

Fig. 1 Bayesian analysis of 29 canine mtDNA genomes. Phylogram constructed using a GTR + G + I model in BEAST 1.7.4, assuming a strict clock and a coalescent model with constant population size. Posterior probability values are reported above nodes and values less than 0.6 are not shown, except for the NGSD/SE lineage node that is of particular interest. Italics indicate samples with discordant mtDNA and nuclear DNA phylogenies. The *scale bar* indicates units of time estimated using a substitution rate of 1.28×10^{-8} mutations^{-1} site^{-1} year^{-1} with a standard deviation of 3.27×10^{-9} (Pang et al. 2009)

K = 2 clustering provides insight into the relationship between SE dingoes and NGSD (Fig. 3). Bayesian clustering analyses identified evidence of genetic subdivision within dingoes, as identified in the mtDNA phylogeny (Fig. 2). Not unexpectedly, there was some evidence of admixture between the dingo populations at nuclear markers. Further a pattern of allele sharing between the SE dingo populations and NGSD was observed, indicating a close relationship (Fig. 2). Indeed when K = 2 SE dingoes and NGSD form a single population cluster (Fig. 3).

Similar population clustering results were observed when structure analyses were repeated excluding two cfOR loci (cfOR0007 and cfOR00034) identified to be under selection (Fig. S2 and Fig. S3). Interestingly, these two cfOR loci are phylogentically informative, with only two divergent alleles observed at each of these genes. A

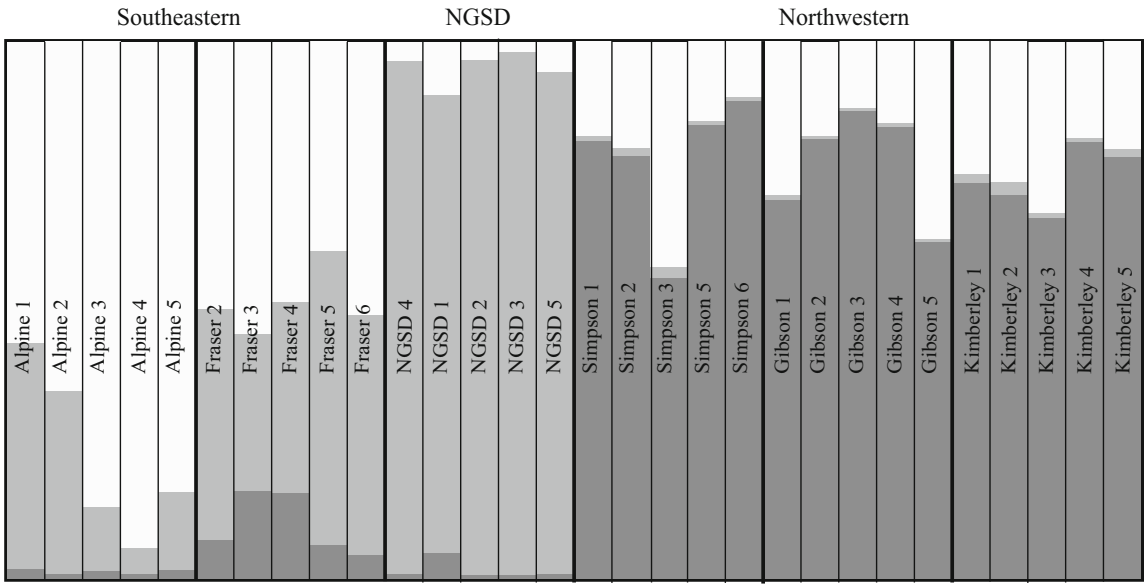

Fig. 2 Bayesian population clustering analysis of 30 canine nuclear haplotypes (across thirteen nuclear loci). Average Q-plot for K = 3 constructed in DISTRUCT v1.1 (Rosenberg 2004). *Each column* represents an individual and the proportion population cluster identity. Population clusters are represented by *colours*: *white* for southeastern, *light grey* for New Guinea Singing Dog and *dark grey* for northwestern

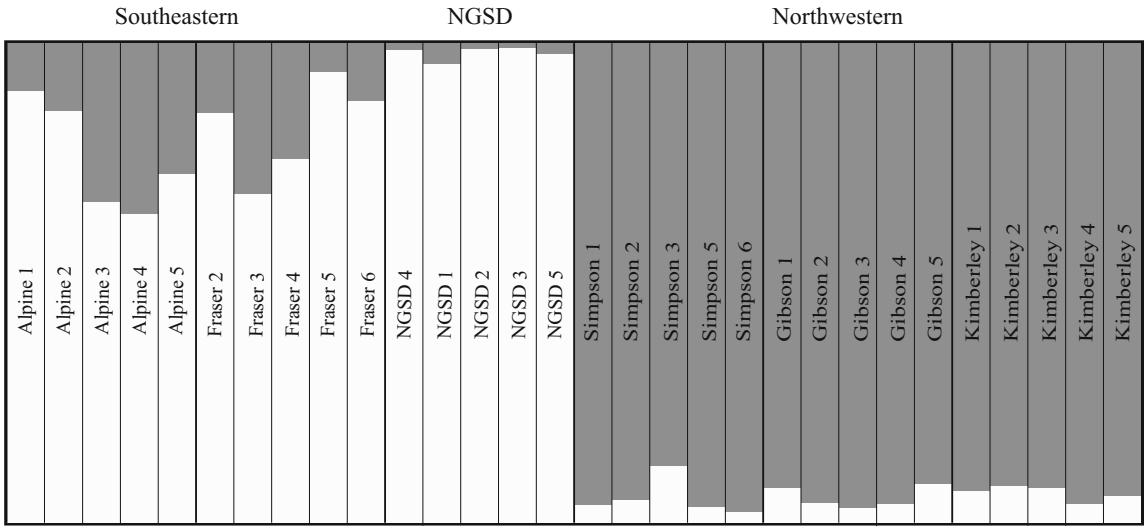

Fig. 3 Bayesian population clustering analysis of 30 canine nuclear haplotypes (across thirteen nuclear loci). Average Q-plot for K = 2 constructed in DISTRUCT v1.1 (Rosenberg 2004). *Each column* represents an individual and the proportion population cluster identity. Population clusters are represented by *colours*: *white* for southeastern/NGSD and *dark grey* for northwestern

contingency table analysis, comparing the distribution of divergent cfOR alleles between the two mtDNA lineages, at cfOR0007 ($\chi^2_2 = 11.7$; $p = 0.003$) and cfOR0034 ($\chi^2_2 = 6.19$; $p = 0.045$) indicates that there is a non-random distribution of these olfactory nuclear haplotypes between the two dingo mtDNA lineages. There is stronger population subdivision evident when all the nuclear genes are included in the analysis because the two genes under selection (cfOR0007 and cfOR0034) are strongly divergent between the two dingo lineages. These data provide further evidence of population subdivision in dingoes.

Statistical analyses

MtDNA

F_{ST} values corroborate the phylogenetic pattern of differentiation observed between the SE lineage and NW

lineage. Values were also high between the Fraser Island and Alpine populations (Table 1). This is likely because Fraser Island is geographically isolated.

We calculated Tajima's *D* for all dingoes and for each mtDNA lineage. When measures of neutrality were calculated for each lineage, the SE lineage had a positive but non-significant Tajima's *D* value whilst the NW lineage had a negative but non-significant Tajima's *D* value (Table 2). Fu and Li's *F** results were not significant but were in the same direction as the Tajima's *D* findings (Table 2). The differing test values between the mtDNA lineages provide initial evidence that the lineages may have been subject to different demographic processes.

Nuclear

To investigate patterns of nuclear variation we conducted F_{ST} calculations and neutrality tests. F_{ST} values were calculated for each individual nuclear gene region and then averaged across all 13 regions to give a "multi locus" view (Table 1). These F_{ST} values corroborate the mtDNA phylogeny and nuclear clustering analysis (Figs. 1, 2, 3). Multi locus F_{ST} values suggest moderate differentiation between the Alpine and Fraser populations but high differentiation between the southeastern populations and the Kimberley, Gibson and Simpson dingo populations. Similarly, the Alpine and Fraser populations are slightly less divergent from the NGSD population than the Kimberley, Gibson and Simpson populations according to the F_{ST} calculations. All the dingo and NGSD populations are highly differentiated from the Indonesian dog based on F_{ST} values. The Simpson population exhibits slightly lower F_{ST} values than the other NW lineage populations suggesting there may be ongoing gene flow into this population (Table 1).

For the 13 nuclear loci, we calculated Tajima's *D* values based upon mitochondrial lineage (i.e. divided into SE versus NW lineages) and observed that there is a general trend for the SE lineage to have positive values, whilst the NW lineage has negative values (Table 2). Two particularly interesting genes that appear to be under selection in the NW lineage were cfOR0007 and cfOR0034. These olfactory genes showed significant positive Tajima's *D* and Fu and Li's *F** values, suggesting balancing selection at these loci, despite the general trend for the lineage to exhibit negative values (Table 2).

Estimating divergence time and substitution rate of mtDNA

We estimated mtDNA divergence implementing a tMRCA statistic for each lineage using the substitution rate obtained from the dog whole mtDNA calibrated with the wolf-coyote fossil divergence by Pang et al. (2009) (Table 3). This substitution rate is likely an underestimate due to the ancient calibration point implemented by Pang et al. (2009); as such divergence estimates should be regarded as maximum values. Under this scenario, estimates of mtDNA divergence time between the SE lineage (and NGSD) and the NW lineage of dingoes are at least 25,400 (25,473–93,806, 95 % HPD) years BP. A fossil-calibrated divergence estimate of the NW dingo lineage is at least 12,300 (12,330–47,634, 95 % HPD) years BP and the SE lineage is at least 9700 (9765–46,889, 95 % HPD) years BP (Fig. 1; Table 3).

In a complimentary analysis, we calculated divergence estimates between oceanic dog and dingo lineages using a more conservative tip-calibrated substitution rate estimated from the Thalmann et al. (2013) dataset (Table 3). Divergence estimates were considerably lower. A more conservative estimate of the divergence time between the SE lineage and NGSD from the NW lineage is 8300 years BP (5742–11,663, 95 % HPD). A conservative divergence estimate of the NW dingo lineage is 4100 years BP (2737–5925, 95 % HPD) and the SE lineage is 3900 years BP (2134–6061, 95 % HPD). The NGSD is estimated to have diverged from the SE dingo lineage 7800 years BP (5184–11,071, 95 % HPD) (Table 3).

Discussion

Phylogenetic analyses of the whole mtDNA provide evidence of at least two subdivided lineages of dingo in Australia. Bayesian clustering analyses based on thirteen

Table 1 F_{ST} values for MtDNA (bottom) and nuclear gene regions (averaged) (top)

	Alpine	Fraser	Gibson	Kimberley	Simpson	NGSD	Indonesia
Alpine	–	0.14	0.23	0.26	0.19	0.21	0.24
Fraser	0.57	–	0.20	0.31	0.13	0.17	0.44
Gibson	0.43	0.75	–	0.06	0.01	0.32	0.42
Kimberley	0.49	0.82	0.17	–	0.12	0.39	0.47
Simpson	0.56	0.88	0.11	0.30	–	0.29	0.36
NGSD	0.46	1.00	0.54	0.67	0.77	–	0.46
Indonesia	0.51	0.86	0.57	0.65	0.72	0.42	–

Table 2 Neutrality test results for mitochondrial and nuclear gene regions

	Gene	NW Lineage		NGSD		SE Lineage	
		Tajima's *D*	Fu and Li's *F**	Tajima's *D*	Fu and Li's *F**	Tajima's *D*	Fu and Li's *F**
MtDNA		−1.68	−2.21	–	–	1.16	0.88
Average (nuclear DNA)		−0.32	−0.35	−0.9	−1.03	0.29	0.65
DLA	DQA1	−0.02	0.02	–	–	−0.36	1.03
	DQB1	−1.08	−1.36	–	–	0.2	1.44
	DRB1	0.55	1.14	–	–	0.91	1.69*
Olfactory receptor	cfOR0007	3.18***	2.45**	−2.01**	−2.56**	–	–
	cfOR0011	−1.72	−1.11	−1.74*	−2.18	1.3	1.54
	cfOR0034	2.61***	2.22**	–	–	1.75	1.83**
	cfOR0123	−0.59	0.17	−0.64	−0.18	−0.71	0.2
	cfOR0184	−0.63	−1.45	–	–	0.49	0.71
	cfOR14A11	−2.34***	−4.14**	−1.56	−1.93	−0.46	−0.95
	DOPRH07	−0.54	0.83	–	–	–	–
Coat Colour	CBD103	−1.05	−0.1	–	–	–	–
	ASIP	−0.72	−0.47	–	–	0.63	0.94
	MC1R	−0.51	−0.87	1.46	1.69	−1.74[#]	−2.15

– Indicates neutrality statistics could not be calculated as no polymorphisms were observed within the population/lineage at this gene region

[#] $0.10 < p < 0.05$; * $p < 0.05$; ** $p < 0.02$; *** $p < 0.001$

Table 3 Estimates of divergence times of dingo lineages

	Pang et al. (2009) substitution rate		Thalmann et al. (2013) substitution rate	
	tMRCA divergence estimate median[a]	tMRCA divergence estimates (95 % HPD)[a]	tMRCA divergence estimate median[a]	tMRCA divergence estimates (95 % HPD)[a]
MRCA dingoes, NGSD and Indonesian dogs	68,790	30,380–107,200	9767	6953–12,978
MRCA dingoes and NGSD	59,640	25,473–93,806	8327	5742–11,663
MRCA NGSD and SE Lineage	55,522	22,723–88,321	7831	5184–11,071
MRCA NW lineage	29,982	12,330–47,634	4183	2737–5925
MRCA SE lineage	28,327	9765–46,889	3969	2134–6061

Pang et al. (2009) rate is 1.28×10^{-8} mutations^{-1} site^{-1} year^{-1} with a standard deviation of 3.27×10^{-9} as compared to the Thalmann et al. (2013) rate of 7.7027×10^{-8} mutations^{-1} site^{-1} year^{-1} with a standard deviation of 5.4848×10^{-9}

[a] Years before present

nuclear loci corroborate this genetic subdivision pattern. Based on their biogeographic distribution we call these lineages the SE and NW lineages (Figs. 1, 2). This is an important finding given the current strong persecution of the dingo in SE Australia and suggests that management and conservation plans need to incorporate information concerning the current population structure of the dingo. Further there is evidence that perhaps dingoes were not brought to Australia as part of the Neolithic expansion and there may have been multiple immigrations.

Patterns of population subdivision are corroborated by F_{ST} values (Table 1). There is evidence of strong differentiation between SE and NW dingo populations at both mitochondrial and nuclear loci. Neutrality test results suggest that the NW lineage may be experiencing population expansion whilst the SE lineage could be suffering population decline or contraction (Table 2). The SE lineage does appear to have a more restricted distribution and plausibly, the NW lineage is, or has been, expanding into the SE lineage's distribution. This observation could also

be the result of divergence between the Alpine and Fraser Island populations. Ecologically relevant factors plausibly impacting dingo lineage distribution patterns may include environmental gradients (Musiani et al. 2007; Stenseth et al. 2004) and neonatal dispersal (Sacks et al. 2004) or prey utilisation (Carmichael et al. 2001; Musiani et al. 2007). In Australia, the establishment of a "dingo proof fence" in the southeastern corner of the continent may influence the movement of contemporary dingoes; however its erection between 1885 and 1950 seems likely too recent to be responsible for the geographical patterns observed in this study. More extensive biogeographic sampling, particularly in Northern Queensland, is needed to resolve the biogeographic distributions and possible introduction routes of the dingo lineages across Australia.

Evidence gathered during this study strongly suggests that inclusion of NGSD samples is necessary to fully investigate the evolutionary history of the Australian dingo. Whole mtDNA genome analyses suggest that the NGSD and the SE lineage of dingoes are distinct but closely related. In the mtDNA phylogeny posterior support for the node is low (Fig. 1). Nuclear clustering analysis provides additional evidence of a close relationship between the NGSD and SE lineage of dingoes, because SE dingoes appear to share nuclear alleles with NGSD at a higher level than NW dingoes (Figs. 2, 3). Y chromosome data corroborates a close relationship between dingoes and NGSD (Ardalan et al. 2012; Sacks et al. 2013). Archaeological and ethnographic evidence suggests that the NGSD once inhabited all of Papua New Guinea but were restricted to the mountains by the arrival of agriculture approximately 5500 years BP (Koler-Matznick et al. 2004). Morphologically the NGSD is similar to the dingo but can be differentiated on the basis of their smaller size and skull morphology (Koler-Matznick et al. 2004). However, it is possible that the mitochondrial phylogeny (Fig. 1) is not representative of the evolutionary relationship between dingoes and NGSD; wider sampling of the autosomal genome may be needed to illuminate the relationship between dingoes and NGSD (Cairns 2015). Increased sampling of NGSD from additional captive populations with a less constrained maternal background, or ideally wild and historical samples, may help further elucidate the relationships between dingo lineages and NGSD.

The close relationship of the SE lineage with the NGSD suggests the divergence between dingo lineages occurred before dispersal into modern Australia and/or outside Australia, plausibly on Sahul (the landmass once incorporating Australia and Papua New Guinea). There are two plausible hypotheses to explain the dingoes' population structure in Australia, first that dingoes immigrated into Australia twice, and second that dingoes have undergone lineage sorting after an initial single introduction (of a heterogeneous population containing both lineages). If two immigrations occurred it is likely that these were the result of dingoes expanding into Australia via the land bridge between Papua New Guinea and Australia, although seafaring introductions cannot be rejected. The hypothesis that dingoes immigrated twice is supported by at least two lines of evidence unless the founding population was small. F_{ST} results corroborate the presence of two geographically subdivided dingo lineages in Australia (Table 1). F_{ST} values also indicate strong geographic population differentiation particularly within the SE lineage due to the geographic isolation of the Fraser Island population.

The hypothesis of two dingo immigrations is supported by Y chromosome data. Ardalan et al. (2012) observed the presence of two distinct paternal lineages, H3 and H60, with an East to West biased distribution. Research on the Y chromosome by Sacks et al. (2013) also supports the idea of an East to West biased distribution of these same lineages. Sacks and colleagues found that the H60 paternal lineage is more similar to Taiwanese dogs than to Island South East Asian dogs suggesting they split outside of Australia (Sacks et al. 2013). An alternative hypothesis is that the observed biogeographical pattern is the result of limited gene flow between populations and genetic drift rather than divergent immigration histories.

There is evidence of admixture between the SE and NW populations according to the nuclear clustering analyses, but little evidence in the mtDNA phylogeny (Figs. 1, 2, 3). This is not unexpected given the larger effective population size, diploid inheritance and higher recombination rates of the nuclear genome compared to the mitochondrial genome. The Fraser Island population appears to be particularly interesting in that it clusters predominately with the SE lineage, carries a high proportion of shared alleles with NGSD and has some evidence of admixture with the NW lineage (Figs. 2, 3). This warrants further investigation into the population history of Fraser Island dingoes. One inconsistency between the mtDNA phylogeny and nuclear clustering is Alpine 5; a dingo collected in the Australian Alpine region that phylogenetically groups with the NW lineage according to the mtDNA phylogeny but clusters with the SE lineage in the nuclear clustering analysis. Plausibly, Alpine 5 is the result of dispersal into southeastern Australia. This is not unexpected as dingoes can be highly mobile (Corbett 1995; Fleming et al. 2001; Thomson et al. 1992). Indeed, differences in male and female dispersal rates or ranging distances have been observed in dingoes (Fleming et al. 2001; Thomson et al. 1992) but it is not known if dingoes harbouring the different mtDNA types have any behavioural or ecological differences.

Next, we employed the whole mtDNA genome data to estimate the time the lineages diverged. It is important to consider that mtDNA data provides a single estimate of the

maternal population history of dingoes, which may not be reflective of the entire populations history. As such, divergence times derived from mtDNA data should be treated with caution. Importantly, divergence time between mitochondrial lineages may not be reflective of the date dingoes arrived Australia. We employed the published estimate of the whole mtDNA substitution rate of canids using the coyote-wolf fossil divergence time (Pang et al. 2009). If this substitution rate is correct, analyses of 25 dingoes from five populations suggest that the SE dingo lineage and the NGSD mtDNA diverged in the ancient continent of Sahul more than 22,700 years BP. Indeed, the most conservative estimate of the tMRCA to the NW lineage is about 12,300 years and to the SE lineage is 9700 years (Table 3). These dates are well before to the Neolithic expansion, which occurred approximately 5500 years BP (McEvoy et al. 2010; Milham and Thompson 1976; van Holst Pellekaan 2013). One possible explanation for these results is that the interspecific substitution rate derived from the coyote-wolf diverge is not appropriate for dating divergence within dingoes. However, whilst the divergence of these lineages is much older than expected they are concordant with molecular divergence estimates based on whole genome sequencing which suggest that wolves and dogs diverged greater than 27,000 years BP (Skoglund et al. 2015; Wang et al. 2013, 2016).

A tip-calibrated substitution rate, employed in a parallel analysis, provides more conservative estimates of the molecular divergence times (Table 3). The two dingo lineages are estimated to have split 8300 years BP and the mean tip-calibrated estimate for the divergence between the SE dingo lineage and the NGSD is 7800 years BP. These dating estimates suggest that it is plausible dingoes and the NGSD diverged in Sahul before dingoes ultimately spread into Australia through the land bridge. One significant consideration with this tip-calibrated substitution rate is that no calibration points from Asia were included in the dataset. This is a particular concern given the dingoes' Asian origin and the complex evolutionary history of dogs in South East Asia (Oskarsson et al. 2011; Sacks et al. 2013; Savolainen et al. 2004). It is important to note that divergence estimates lower than 3500 years conflict with the known minimum arrival time of the dingo set by the fossil record (Macintosh 1975). Future molecular dating estimates should endeavour to incorporate a more diverse geographical range of fossil calibration points and specimen.

There is considerable evidence that dingoes are not a modern introduction to Australia. Four independent lines of evidence suggest that dingoes immigrated into Australia prior to the Neolithic expansion. First, Y chromosome data indicates that dingoes are an older dog lineage that those in surrounding Island South East Asia, suggesting dingoes are the result of an earlier dog radiation, plausibly prior to the Neolithic expansion (Sacks et al. 2013). Second, despite uncertainty regarding the timing of dingo divergence, there is increasing genetic evidence that suggests that dingoes and dogs evolved prior to the rise of agriculture. Evidence that dingoes do not carry duplications of AMY2B, which most domestic dogs carry, indicate that dingoes are unlikely to have been associated with agricultural cultures such as the Neolithic (Arendt et al. 2016; Freedman et al. 2014). Third, a recently discovered rock art painting of a dingo from Nawarla Gabarnmang in the Northern Territory, suggests dingoes may have arrived in Australia earlier than previously hypothesised (David et al. 2013).

Finally, the Neolithic expansion into South East Asia, Papua New Guinea and Polynesia is characterised by human gene flow, introduction of agriculture and the presence of cultural items such as pigs, chickens and domestic dogs (Bocquet-Appel 2011; Haak et al. 2010; Karafet et al. 2005; Larson et al. 2007; Oskarsson et al. 2011; Sacks et al. 2013). However, cultural items associated with the Neolithic such as pigs and chickens were not brought to Australia until European colonisation in 1788 (Larson et al. 2007, 2010; Oskarsson et al. 2011). There is no evidence of gene flow between Neolithic or East Asian human populations and Indigenous Australians (McEvoy et al. 2010; van Holst Pellekaan 2013). Further, Agriculture did not occur in Australia until the European colonisation in 1788. This ultimately suggests that the Neolithic expansion did not reach Australia and was consequently not responsible for bringing dingoes to Australia (Fillios and Taçon 2016). There is, however, one major issue with the hypothesis that dingoes colonised Australia before the Neolithic expansion: there are currently no dingoes in the fossil record prior to 3500 years BP. One possible explanation could be that dingo fossils have not been well preserved in Northern Australia and Papua New Guinea. Certainly, there is a general paucity of fossils in these regions due to soil acidity and tropical climatic conditions (Kidwell and Flessa 1996; Tappen 1994). We suggest it is unlikely that the mtDNA diverged long before the dingo colonised Australia because the lineages are clearly geographically subdivided in Australia.

Conclusions

Mitochondrial and nuclear data presented in this study, and published in Y chromosome studies (Ardalan et al. 2012; Sacks et al. 2013) suggest the presence of at least two strongly subdivided lineages of dingo in Australia. This is of great importance to the conservation and management of the dingo in Australia. Populations representing both

lineages should be protected and breeding programs should be designed incorporating this information. The legislative status of the dingo needs to be updated from feral pest, and management practices may need to be adapted with an emphasis on conservation rather than exclusion and/or eradication. We hypothesise that dingoes immigrated to Australia at least twice. Our data suggest that plausibly dingoes and the NGSD diverged outside modern Australia and that the two lineages of dingo diverged before immigration into Australia. The two dingo lineages may have immigrated over the land bridge between Australia and Papua New Guinea. Genetic evidence, including molecular dating, suggests that potentially the human Neolithic expansion was not responsible for introducing the dingo and NGSD to Oceania.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. This research was approved by the Animal Care and Ethics Committee of University of NSW (Permit Number: 12/36B).

References

Anderson TM et al (2009) Molecular and evolutionary history of melanism in North American gray wolves. Science 323:1339–1343. doi:10.1126/science.1165448

Ardalan A, Oskarsson M, Natanaelsson C, Wilton A, Ahmadian A, Savolainen P (2012) Narrow genetic basis for the Australian dingo confirmed through analysis of paternal ancestry. Genetica 140:65–73. doi:10.1007/s10709-012-9658-5

Arendt M, Cairns KM, Ballard JWO, Savolainen P, Axelsson E (2016) Diet adaptation in dog reflects spread of prehistoric agriculture. Heredity. doi:10.1038/hdy.2016.48

Bocquet-Appel J-P (2011) When the world's population took off: the springboard of the neolithic demographic transition. Science 333:560–561. doi:10.1126/science.1208880

Brown RP, Yang Z (2011) Rate variation and estimation of divergence times using strict and relaxed clocks. BMC Evol Biol 11:271

Cairns KM (2015) Population differentiation in the dingo: biogeography and molecular ecology of the Australian Native Dog using maternal, paternal and autosomal genetic markers. University of New South Wales, Sydney

Carmichael LE, Nagy JA, Larter NC, Strobeck C (2001) Prey specialization may influence patterns of gene flow in wolves of the Canadian Northwest. Mol Ecol 10:2787–2798. doi:10.1046/j.0962-1083.2001.01408.x

Carthey AJR, Banks PB (2012) When does an alien become a native species? A vulnerable native mammal recognizes and responds to its long-term alien predator. PLoS ONE 7:e31804. doi:10.1371/journal.pone.0031804

Corbett LK (1995) The dingo in Australia and Asia. University of NSW Press, Sydney

Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. Nat Methods 9:772–772. http://www.nature.com/nmeth/journal/v9/n8/abs/nmeth.2109.html—supplementary-information

David B et al (2013) A 28,000 year old excavated painted rock from Nawarla Gabarnmang, northern Australia. J Archaeol Sci 40:2493–2501. doi:10.1016/j.jas.2012.08.015

Davis E (2001) Legislative issues relating to control of dingoes and other wild dogs in New South Wales. 1. Approaches to future management. In: Dickman CR, Lunney D (eds) A symposium on the Dingo. Royal Zoological Society of New South Wales, Sydney

Diamond J (2002) Evolution, consequences and future of plant and animal domestication. Nature 418:700–707

Drummond AJ, Suchard MA, Xie D, Rambaut A (2012) Bayesian phylogenetics with BEAUti and the BEAST 1.7. Mol Biol. doi:10.1093/molbev/mss075

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software structure: a simulation study. Mol Ecol 14:2611–2620. doi:10.1111/j.1365-294X.2005.02553.x

Excoffier L, Lischer HEL (2010) Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows Mol. Ecol Res 10:564–567. doi:10.1111/j.1755-0998.2010.02847.x

Fillios MA, Taçon PSC (2016) Who let the dogs in? A review of the recent genetic evidence for the introduction of the dingo to Australia and implications for the movement of people. J Archaeol Sci Rep. doi:10.1016/j.jasrep.2016.03.001

Fleming P, Corbett LK, Harden R, Thomson P (2001) Managing the impacts of dingoes and other wild dogs. Bureau of Rural Sciences, Canberra

Frantz LAF et al (2016) Genomic and archaeological evidence suggest a dual origin of domestic dogs. Science 352:1228–1231. doi:10.1126/science.aaf3161

Freedman AH et al (2014) Genome sequencing highlights the dynamic early history of dogs. PLoS Genet 10:e1004016. doi:10.1371/journal.pgen.1004016

Fu YX (1997) Statistical tests of neutrality of mutations against population growth. Hitchhiking Backgr Sel Genet 147:915–925

Gibbons A (2001) The peopling of the Pacific. Science 291:1735–1737. doi:10.1126/science.291.5509.1735

Gollan K (1984) The Australian dingo: in the shadow of man. In: Archer M, Clayton G (eds) Vertebrate zoogeography and evolution in Australasia. Hesperian Press, Carlisle

Haak W et al (2010) Ancient DNA from european early neolithic farmers reveals their near eastern affinities. PLoS Biol 8:e1000536. doi:10.1371/journal.pbio.1000536

Ho SYW, Lanfear R, Bromham L, Phillips MJ, Soubrier J, Rodrigo AG, Cooper A (2011) Time-dependent rates of molecular evolution. Mol Ecol 20:3087–3101. doi:10.1111/j.1365-294X.2011.05178.x

Ho SYW, Larson G (2006) Molecular clocks: when times are a-changin'. Trends Genet 22:79–83

van Holst Pellekaan S (2013) Genetic evidence for the colonization of Australia. Quat Int 285:44–56. doi:10.1016/j.quaint.2011.04.014

Hubisz MJ, Falush D, Stephens M, Pritchard JK (2009) Inferring weak population structure with the assistance of sample group information. Mol Ecol Res 9:1322–1332. doi:10.1111/j.1755-0998.2009.02591.x

Jakobsson M, Rosenberg NA (2007) CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. Bioinformatics 23:1801–1806

Karafet TM et al (2005) Balinese Y-chromosome perspective on the peopling of Indonesia: genetic contributions from pre-neolithic hunter–gatherers, Austronesian farmers, and Indian traders. Hum Biol 77:93–114

Kennedy LJ, Angles JM, Barnes A, Carmichael LE, Radford AD, Ollier WER, Happ GM (2007) DLA-DRB1, DQA1, and DQB1 alleles and haplotypes in North American gray wolves. J Hered 98:491–499. doi:10.1093/jhered/esm051

Kidwell SM, Flessa KW (1996) The quality of the fossil record: populations, species, and communities. Annu Rev Earth Planet Sci 24:433–464. doi:10.1146/annurev.earth.24.1.433

Kingman JFC (1982) The coalescent. Stoch Proc Appl 13:235–248. doi:10.1016/0304-4149(82)90011-4

Koler-Matznick J, Brisbin IL, Feinstein M, Bulmer S (2004) An updated description of the New Guinea singing dog (*Canis hallstromi*, Troughton 1957). J Zool 261:109–118. doi:10.1017/S0952836903004060

Larson G et al (2007) Phylogeny and ancient DNA of Sus provides insights into neolithic expansion in Island Southeast Asia and Oceania. PNAS 104:4834–4839. doi:10.1073/pnas.0607753104

Larson G et al (2010) Patterns of East Asian pig domestication, migration, and turnover revealed by modern and ancient DNA. PNAS 107:7686–7691. doi:10.1073/pnas.0912264107

Letnic M, Greenville A, Denny E, Dickman CR, Tischler M, Gordon C, Koch F (2010) Does a top predator suppress the abundance of an invasive mesopredator at a continental scale? Global Ecol Biogeogr 20:343–353

Letnic M, Ritchie EG, Dickman CR (2012) Top predators as biodiversity regulators: the dingo Canis lupus dingo as a case study. Biol Rev 87:390–413. doi:10.1111/j.1469-185X.2011.00203.x

Librado P, Rozas J (2009) DNAsp v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25:1451–1452

Macintosh NWG (1975) The origin of the dingo: an enigma. In: Fox MW (ed) The Wild Canids—their systematics, behavioural ecology and evolution. Behavioral Science. Van Nostrand Reinhold Company, New York, pp 87–106

McEvoy BP, Lind JM, Wang ET, Moyzis RK, Visscher PM, van Holst Pellekaan SM, Wilton AN (2010) Whole-genome genetic diversity in a sample of Australians with deep aboriginal ancestry. Am J Hum Genet 87:297–305. doi:10.1016/j.ajhg.2010.07.008

Milham P, Thompson P (1976) Relative antiquity of human occupation and extinct fauna at madura cave. Southeastern Western Australia Mankind 10:175–180. doi:10.1111/j.1835-9310.1976.tb01149.x

Moseby KE, Neilly H, Read JL, Crisp HA (2012) Interactions between a top order predator and exotic mesopredators in the Australian rangelands. Int J Ecol. doi:10.1155/2012/250352

Musiani M et al (2007) Differentiation of tundra/taiga and boreal coniferous forest wolves, coat color and association with migratory caribou. Mol Ecol 16:4149

Oskarsson MCR, Klütsch CFC, Boonyaprakob U, Wilton A, Tanabe Y, Savolainen P (2011) Mitochondrial DNA data indicate an introduction through Mainland Southeast Asia for Australian dingoes and Polynesian domestic dogs. Proc R Soc B. doi:10.1098/rspb.2011.1395

Pang J-F et al (2009) mtDNA data indicate a single origin for dogs south of Yangtze river, less than 16,300 years ago, from numerous wolves. Mol Biol Evol 26:2849–2864

Rambaut A, Drummond A (2007) Tracer v1.5 edn. http://beast.bio.ed.ac.uk/Tracer

Randall D, Pollinger J, Argaw K, Macdonald D, Wayne R (2010) Fine-scale genetic structure in Ethiopian wolves imposed by sociality, migration, and population bottlenecks. Cons Gen 11:89–101. doi:10.1007/s10592-009-0005-z

Ripple WJ et al (2014) Status and ecological effects of the world's largest carnivores. Science 343. doi:10.1126/science.1241484

Robin S et al (2009) Genetic diversity of canine olfactory receptors. BMC Genom. doi:10.1186/1471-2164-10-21

Rosenberg NA (2004) DISTRUCT: a program for the graphical display of population structure. Mol Ecol Notes 4:137–138

Sacks BN, Brown SK, Ernest HB (2004) Population structure of California coyotes corresponds to habitat-specific breaks and illuminates species history. Mol Ecol 13:1265–1275. doi:10.1111/j.1365-294X.2004.02110.x

Sacks BN, Brown SK, Stephens D, Pedersen NC, Wu J-T, Berry O (2013) Y chromosome analysis of dingoes and Southeast Asian village dogs suggests a Neolithic continental expansion from Southeast Asia followed by multiple Austronesian dispersals. Mol Biol Evol 13:1265–1275. doi:10.1093/molbev/mst027

Savolainen P, Leitner T, Wilton AN, Matisoo-Smith E, Lundeberg J (2004) A detailed picture of the origin of the Australian dingo, obtained from the study of mitochondrial DNA. PNAS 101:12387–12390

Shannon LM et al (2015) Genetic structure in village dogs reveals a Central Asian domestication origin. PNAS 112:13639–13644. doi:10.1073/pnas.1516215112

Skoglund P, Ersmark E, Palkopoulou E, Dalén L (2015) Ancient wolf genome reveals an early divergence of domestic dog ancestors and admixture into high-latitude breeds. Curr Biol 25:1515–1519. doi:10.1016/j.cub.2015.04.019

Stenseth NC et al (2004) Snow conditions may create an invisible barrier for lynx. PNAS 101:10632–10634

Stephens D (2011) The molecular ecology of Australian wild dogs: hybridisation, gene flow and genetic structure at multiple geographic scales. PhD, University of Western Australia

Tacher S, Quignon P, Rimbault M, Dreano S, Andre C, Galibert F (2005) Olfactory receptor sequence polymorphism within and between breeds of dogs. J Hered 96:812–816. doi:10.1093/jhered/esi113

Tajima F (1989) Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123:585–595

Tappen M (1994) Bone weathering in the tropical rain forest. J Archaeol Sci 21:667–673. doi:10.1006/jasc.1994.1066

Thalmann O et al (2013) Complete mitochondrial genomes of ancient Canids suggest a European origin of domestic dogs. Science 342:871–874. doi:10.1126/science.1243650

Thomson P, Rose K, Kok N (1992) The behavioural ecology of dingoes in north-western Australia. VI. Temporary extraterritorial movements and dispersal. Wildl Res 19:585–595. doi:10.1071/WR9920585

Von Holdt BM et al (2010) Genome-wide SNP and haplotype analyses reveal a rich history underlying dog domestication.

Nature 464:898–902. doi:http://www.nature.com/nature/journal/vaop/ncurrent/suppinfo/nature08837_S1.html

Wallach AD, Johnson CN, Ritchie EG, O'Neill AJ (2010) Predator control promotes invasive dominated ecological states. Ecol Lett 13:1008–1018. doi:10.1111/j.1461-0248.2010.01492.x

Wang G et al (2013) The genomics of selection in dogs and the parallel evolution between dogs and humans. Nat Commun 4:1860. doi:10.1038/ncomms2814

Wang G-D et al (2016) Out of southern East Asia: the natural history of domestic dogs across the world. Cell Res 26:21–33. doi:10.1038/cr.2015.147

Wilton A (2001) DNA methods of assessing australian dingo purity. In: Dickman CR, Lunney D (eds) A Symposium on the Australian dingo. Royal Zoological Society of New South Wales, Sydney, pp 49–55