

# A detailed picture of the origin of the Australian dingo, obtained from the study of mitochondrial DNA

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To determine the origin and time of arrival to Australia of the dingo, 582 bp of the mtDNA control region were analyzed in 211 Australian dingoes sampled in all states of Australia, 676 dogs from all continents, and 38 Eurasian wolves, and 263 bp were analyzed in 19 pre-European archaeological dog samples from Polynesia. We found that all mtDNA sequences among dingoes were either identical to or differing by a single substitution from a single mtDNA type, A29. This mtDNA type, which was present in >50% of the dingoes, was found also among domestic dogs, but only in dogs from East Asia and Arctic America, whereas 18 of the 19 other types were unique to dingoes. The mean genetic distance to A29 among the dingo mtDNA sequences indicates an origin  $\approx$ 5,000 years ago. From these results a detailed scenario of the origin and history of the dingo can be derived: dingoes have an origin from domesticated dogs coming from East Asia, possibly in connection with the Austronesian expansion into Island Southeast Asia. They were introduced from a small population of dogs, possibly at a single occasion, and have since lived isolated from other dog populations.

The question of the origin of the Australian dingo has been the subject of much public interest ever since the arrival of Europeans to Australia in the 18th century (1, 2). The facts that the dingo was the only large placental mammal except humans on the continent and that it is a canid closely resembling domestic dogs, yet wild, gave rise to debate about its origin, especially during the first half of the 20th century. Later archaeological and morphological studies have suggested a relatively late introduction of the dingo and a close relationship to the domestic dog. However, the precise ancestry, place of origin, and time of arrival in Australia of the dingo have not been determined, nor whether, on its arrival, it was a domesticated or half-domesticated animal becoming feral or a truly wild dog.

The dingo was present throughout the Australian mainland by the time of the arrival of Europeans (3). Mostly it lived as a wild animal, but some groups of Aborigines used dingoes in a semidomestic state as pets or in hunting. However, full domestication of dingoes has proved difficult (1); as a pet, it is generally more independent-minded than dogs. The dingo is similar in general morphology to South Asian domestic dogs (3), and in skeletal morphology it especially resembles Indian pariah dogs and wolves (4, 5). In measures of skull morphology, values for dingoes are between those of dogs and wolves, overlapping with both (3).

In a sparse archaeological record, the earliest substantiated evidence of dingoes is from  $\approx$ 3,500 yr ago (2, 6). Finds are absent in Tasmania, which was separated from Australia by the rise of the sea level  $\approx$ 12,000 yr ago (2). Archaeological data therefore indicates the arrival of dingoes to Australia some time between 3,500 and 12,000 yr ago. To reach Australia from the Asian mainland through the Southeast Asian archipelago, regardless of route, a journey of at least 50 km over open sea was necessary at some point even at low sea level of glacial maxima, and there is no example of any other large terrestrial animal that has made this journey unaided (1, 7). The dingo ancestors were therefore

most probably introduced to Australia with the aid of humans traveling in boats. There are two main hypotheses proposed for the geographic origin of the dingo ancestors. East Asia has been suggested as the origin based on the relative proximity to Australia and the relatively easy access via the islands of the Southeast Asian archipelago (3). Alternatively, based on the close similarity in skeletal anatomy to Indian pariah dogs and wolves, an introduction from India by maritime peoples has been proposed (5). This theory would be supported by the suggestion that the backed-blade stone-tool technology first appearing in Australia approximately simultaneously with the dingo could have been influenced from India (8), but others argue against an influence from outside Australia for the development of this technology (9). Up to now, mtDNA has been studied in only four dingoes, all having one and the same mtDNA type, which is found also among domestic dogs (10), suggesting an origin for dingoes from domestic dogs.

To obtain a more comprehensive picture of the origin and history of the dingo, we analyzed mtDNA sequences in 211 dingoes and compared them to a world-wide sample of 676 dogs. Detailed questions could be elucidated by: (i) investigating whether dingoes generally have mtDNA sequences similar to those of dogs and thus can be unequivocally shown to originate from domesticated dogs; (ii) estimating the number of mtDNA types introduced and the number of times these introductions occurred; (iii) comparing the mtDNA types found in dingoes with the geographic distribution of types among dogs to estimate the place of origin for the founder animals; and (iv) calculating the genetic divergence among dingo mtDNA types compared with the founder type(s) to estimate the time for the first introduction of dingoes to Australia.

## Materials and Methods

**Samples.** Dingoes ( $n = 211$ ), dogs ( $n = 22$ ), and pre-European archaeological dog ( $n = 19$ ) samples from Polynesia were sequenced in this study and compared with 654 dog and 38 wolf samples used by Savolainen *et al.* (11). For the geographic origin of dingo samples, see Table 1. The origins of the dog samples [for a detailed list of geographic origin and breeds, see Savolainen *et al.* (11)] were Europe ( $n = 207$ ), Africa ( $n = 35$ ), Southwest Asia ( $n = 90$ ), India ( $n = 27$ ), Siberia ( $n = 24$ ), Arctic America ( $n = 25$ ), China/Mongolia/Korea ( $n = 142$ ), Japan ( $n = 96$ ), Vietnam/Cambodia/Thailand ( $n = 18$ ), Indonesia ( $n = 7$ ), Malaysia ( $n = 2$ ), Philippines ( $n = 1$ ), and Highland New Guinea ( $n = 2$ ). Prehistoric archaeological dog samples were from Cook Island ( $n = 2$  dogs from sites dating from 1,000 B.P.), New Zealand ( $n = 13$  from sites dating 700–400 B.P.), and Hawaii ( $n = 4$  from prehistoric sites of unknown age).

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Abbreviation: Myr, million years.

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**Table 1. Number of individuals per mtDNA type for dingoes in Northern Territory (NT), Queensland (Qld), Western Australia (WA), South Australia (SA), Victoria (Vic), and New South Wales (NSW)**

Haplotype	NT	Qld	WA	SA	Vic	NSW	Total
Din1, A29	3	13	9	3	17	67	112
Din2	—	—	2	1	—	2	5
Din3	—	5	—	1	—	—	6
Din4	—	—	9	—	—	—	9
Din5	—	1	—	—	—	—	1
Din6	—	—	1	—	1	—	2
Din8	—	7	—	—	4	9	20
Din9	—	1	5	—	—	—	6
Din10	—	1	—	—	—	—	1
Din11	—	1	—	—	—	—	1
Din12	—	—	—	—	—	1	1
Din13	—	1	—	—	—	1	2
Din14	—	—	—	—	—	8	8
Din15	—	—	1	—	8	8	17
Din16	—	—	—	—	2	10	12
Din17	—	—	2	—	—	—	2
Din18	—	1	—	—	—	2	3
Din19, A9	—	—	—	—	1	—	1
Din20	—	1	—	—	—	—	1
Din21	—	1	—	—	—	—	1
Total	3	33	29	5	33	108	211

Nineteen dingoes were from captivity in zoos, wildlife parks, dingo-conservation groups, and dingo fanciers, and 192 animals were sampled from the wild from 27 regions across the country. The wild animals were chosen based on similarity in appearance to dingoes to exclude as far as possible feral dogs and dog × dingo hybrids. The main areas sampled were the Pilbarra area of Western Australia (25 samples from 10 localities), the north tablelands of New South Wales (20 samples mainly from two national parks), southeastern New South Wales (65 samples from five major localities), and northeastern Victoria (18 samples from two localities). The remaining samples were individual samples from various areas across the country.

**PCR and Sequencing.** DNA extraction, PCR, and sequence analysis of dingo and modern dog samples were performed as described by Savolainen *et al.* (11). Five hundred and eighty-two base pairs, positions 15,458–16,039 of the dog mitochondrial genome (12), were analyzed. Archaeological samples were analyzed as described by Matisoo-Smith *et al.* (13) by using primers L15910 and H16498 [described by DeSalle *et al.* (14)] and sequenced from at least two independent PCRs to validate polymorphic positions. A total of 263 bp, positions 15,458–15,720, was analyzed.

**Phylogenetic Analysis.** The tree in Fig. 1A, was calculated as described (11). Briefly, a neighbor-joining starting tree was calculated by using an HKY nucleotide-substitution model with invariable sites and a  $\gamma$  distribution to describe variable rates across sites that were not unvaried ( $I = 0.7266$  and  $\alpha = 0.6361$ ). The root of the dog/wolf clade was derived by midpoint rooting and a Kishino–Hasegawa maximum-likelihood test of alternative rooting points by using RELL bootstrap (1,000 replicates). The tree was searched further by >2.5 million tree bisection and reconnection iterations without additional improvement. The individual dog clades A–D and F were imploded, because the network structure within the dog clades, caused by a large degree of homoplasy, could not be described accurately by tree bifurcations, as depicted in clade A in Fig. 1B. All established dog clades were retained compared with previous reports (10, 11),

but note that, because of the homoplasy, the order between some of the clades is different. The order between these clades should be regarded as uncertain at this point. It is important, however, that the order did not affect any of the conclusions regarding dog and dingo origins. The minimum-spanning network shown in Fig. 1B was constructed by using ARLEQUIN software (15).

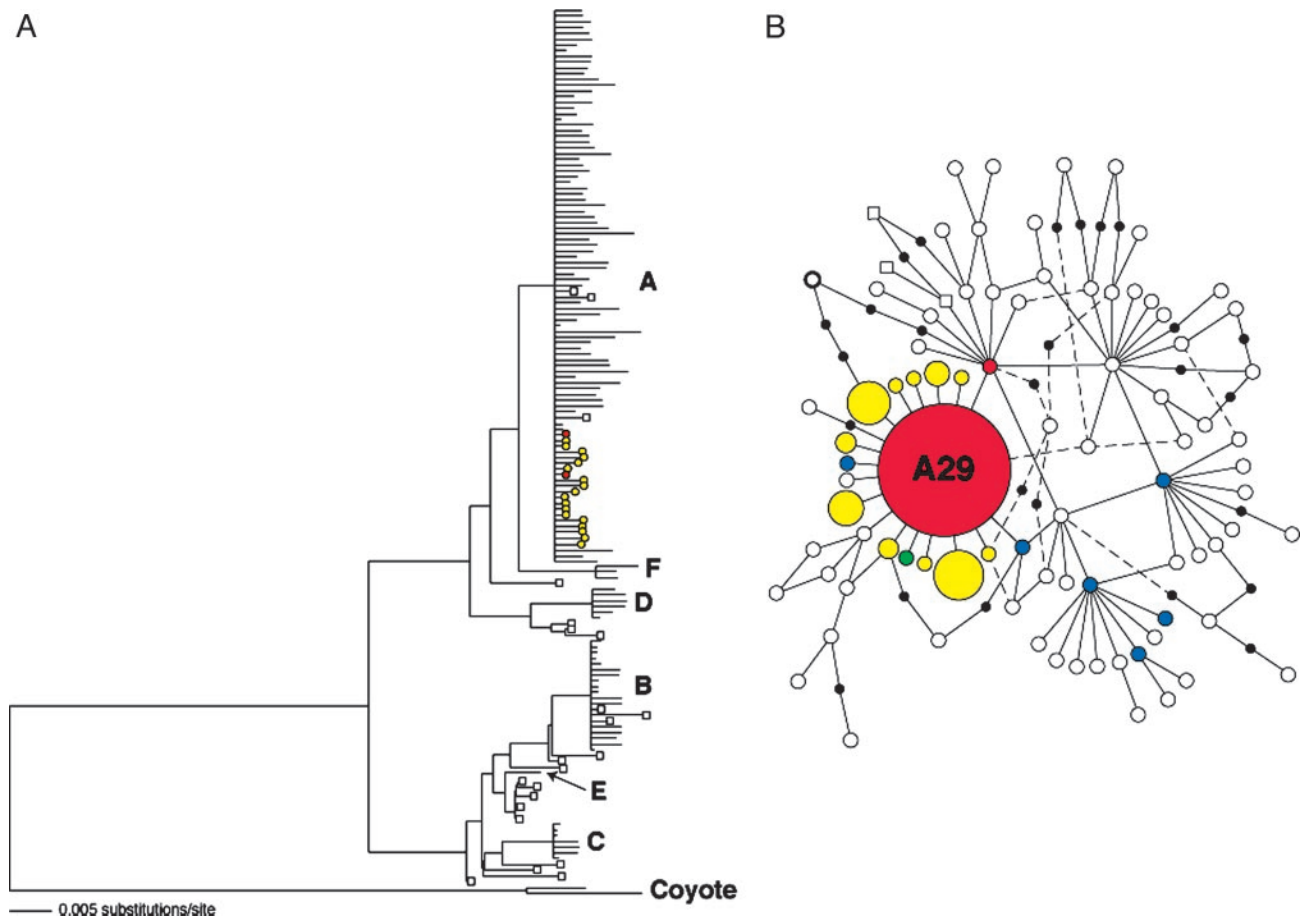
**Calculation of Genetic Distances.** Based on the assumption that A29 was the only founder mtDNA type, an estimate of the time for the introduction of dingoes to Australia was calculated from the mean distance to A29 among dingo sequences. The standard deviation of the distance to A29 was calculated by resampling with replacement (1,000 sets) of all dingo sequences ( $n = 211$ ). The mutation rate of the analyzed region was estimated from the mean genetic distance between the dingo, dog, and wolf mtDNA types and the coyote types in the phylogenetic tree (Fig. 1A) and the assumption of a divergence time between wolves and coyotes of 1 million years (Myr), which is based on a first appearance of wolves  $\approx 700,000$  yr ago and of coyotes  $\approx 1$  Myr ago (16, 17).

## Results

mtDNA was analyzed in 211 Australian dingoes sampled in all states of Australia (Table 1), 676 dogs from all continents, and 38 Eurasian wolves, and 290 bp were analyzed in 19 pre-European archaeological dog samples from Polynesia. The sequence variation among the dingoes was very restricted compared with that of dogs and wolves (Fig. 2, which is published as supporting information on the PNAS web site). Among the 211 dingoes there were 20 mtDNA types differing by at most two substitutions, whereas among 676 dogs there were 114 mtDNA types with a maximum difference of 16 substitutions between mtDNA types. Two of the dingo mtDNA types were identical to dog mtDNA types (din1 = A29; din19 = A9), whereas the other 18 were unique to dingoes.

It has been shown that in a phylogenetic tree of dog and wolf sequences, domestic dog mtDNA types form several separate branches distributed among the wolf mtDNA types, showing that the domestic dog originates from several maternal wolf lines (10, 11). In a phylogenetic tree of dogs, wolves, and dingoes, all the dingo sequences fall into the main clade (A) of dog sequences containing  $\approx 70\%$  of domestic dog mtDNA types (Fig. 1A). A minimum-spanning network of clade A shows the dingo mtDNA types forming a single internal cluster around a central type, A29, found in both dingoes and dogs (Fig. 1B). A29, which was found in 53% of all dingoes, is surrounded by the other 12 (discounting indels) less frequent dingo types, differing by a single substitution, as well as by a number of dog mtDNA types. Among domestic dogs, A29 was found only in East Asia (East Siberia, in 2 of 18 individuals; Japan, 4 of 96; Indonesia, 2 of 7), New Guinea (1 of 2), and Arctic America (6 of 25) (11). Among dogs from the islands surrounding Australia, several mtDNA types other than A29 were found. In Island Southeast Asia (Indonesia,  $n = 7$  dogs; Malaysia  $n = 2$  dogs; the Philippines  $n = 1$  dog), there were seven mtDNA types in addition to A29 (Fig. 1B). Among 19 samples of dogs from Polynesian pre-European archaeological sites, there were two mtDNA types, Arc 1 and Arc 2 (Fig. 2), both different from A29, on Cook Islands (Arc1,  $n = 1$ ; Arc2,  $n = 1$ ), New Zealand (Arc1,  $n = 3$ ; Arc2,  $n = 10$ ), and Hawaii (Arc1,  $n = 2$ ; Arc2,  $n = 2$ ). Interestingly, for the region sequenced for both ancient and contemporary samples, Arc2 is identical to mtDNA type A75, which was found only in two Indonesian samples, whereas Arc1 has a sequence found in a number of widely spread types (11).

The mutation rate for the analyzed region was estimated from the mean genetic distance between the dingo, dog, and wolf mtDNA types and the coyote types in the phylogenetic tree (Fig. 1A) and the assumption of a divergence time between wolves and coyotes of 1 Myr, to 0.0651 substitutions site<sup>-1</sup> Myr<sup>-1</sup> (SD =



**Fig. 1.** Genetic relationships between mtDNA control region sequences (582 bp) from dingoes, domestic dogs, and wolves. (A) Phylogenetic tree showing all dog, wolf, and dingo mtDNA types. Unlabeled leaves denote mtDNA types from domestic dogs, yellow circles denote types unique to dingoes, red circles denote types found in both dingo and dog, and squares denote wolf mtDNA types. The tree is rooted to coyote sequences. Dog clades A–F are indicated with letters. (B) Minimum-spanning network of the main dog clade (clade A). Sequence types (circles) and empty nodes (solid dots) are separated by one mutational step (substitutions; indels are not shown). The mtDNA type indicated by bold lines has four shortest links (with a length of three mutational steps) to other mtDNA types, but two of these are not shown to simplify the figure. Yellow, unique dingo mtDNA type; red, type found in both dingo and dog; blue, type found in dogs in Indonesia, the Philippines, or Malaysia; green, unique New Guinea type. Squares denote wolves. Areas of red and yellow circles are proportional to frequencies among dingoes, but the area of A29 is reduced by 50%.

0.0027). The divergence time is based on paleontological evidence of a first appearance of wolves  $\approx 700,000$  yr ago and of coyotes  $\approx 1$  Myr ago (16, 17) and seems to be the most probable estimate; however, it has not been definitely established, and an earlier date of up to  $\approx 2$  Myr cannot be ruled out (17–19).

The star-like formation of the dingo mtDNA sequences in the minimum-spanning network (Fig. 1B) and the facts that more than half of the dingoes had the central type A29 and all dingo mtDNA sequences but A29 and A9 were unique to dingoes indicate that all dingo mtDNA types originate from A29. A9 was found in a single dingo individual (Table 1), and considering the relatively high degree of homoplasy in the data set, shown by a large homoplasy index for the phylogenetic tree (HI = 0.56), it is probable that this mtDNA type is the result of a parallel mutation. Assuming that A29 was the only founder type, the probable time for the introduction of dingoes to Australia was estimated at 4,600–5,400 yr ago from the mean distance to A29 among dingo sequences (0.190 substitutions; SD = 0.007) and the mutation rate, based on a wolf–coyote split 1 Myr ago. Taking into account the possibility of a split up to 2 Myr ago, the introduction would have happened some time 4,600–10,800 yr ago. The mean distance to A29 among dingo sequences differs considerably between Western Australia and the other parts of Australia (Table 1). This discrepancy is likely caused by random

genetic drift in the Pilbara District population from which most Western Australian samples (25 of 29) were taken, and therefore the mean value for all dingo samples was used.

### Discussion

The position of the dingo mtDNA types in the middle of the dog mtDNA variation, A29 being found among both dogs and dingoes and forming an internal node in clade A surrounded by the other dingo mtDNA types as well as by dog mtDNA types, provides strong evidence that dingoes originated from domesticated dogs. It was shown before that domestic dogs in Eurasia, America, and Africa have a common origin (11, 20), and with this study it is shown that this applies to domesticated dogs on all continents.

The minimum-spanning network of phylogenetic clade A has a complicated pattern of dog haplotypes, caused by a large degree of homoplasy, which shows that the analyzed region is not sufficient for obtaining a phylogenetic resolution of dog mtDNA types. However, the dingo types are well grouped, and the very restricted sequence variation among dingoes, with more than half of the dingoes having mtDNA type A29 and all other mtDNA types forming a star-like formation around A29, and the fact that all other dingo types but A9 were unique to dingoes suggest that all dingo mtDNA types originate from A29. This



indicates that the dingo population was formed either from very few dogs, theoretically as few as a single pregnant female, or from a group of dogs that had radically lost genetic variation through one or several severe bottlenecks on their way from the Asian mainland through Island Southeast Asia. However, the presence of a number of mtDNA types other than A29 on the islands surrounding Australia, both in modern samples and in samples from pre-European archaeological sites, indicates that there were several mtDNA types other than A29 present in the region and points at a single founding event. It also indicates that there have not been any substantial later introductions of dogs to Australia before the arrival of Europeans.

Today there is a large amount of hybridization between dingoes and domestic dogs in eastern Australia, but for this study the wild dingoes were sampled based on similarity in appearance to dingoes to exclude as far as possible feral dogs and dog × dingo hybrids, and the absence among the dingoes of any mtDNA types found in European dog breeds (11) indicates a low degree of hybrids among the sampled dingoes. However, because of the maternal mode of inheritance of mtDNA, hybridization between male dogs and female dingoes would not be identified by these mtDNA analyses. It can be noted that, similar to dingoes, the New Guinean dogs had the mtDNA type A29 and a unique type differing from A29 by one substitution. These so-called New Guinea singing dogs are feral and show some morphological and behavioral similarities to dingoes. A common origin and some gene flow between the two populations is therefore possible.

Among domestic dogs, A29 was found only among East Asian, Island Southeast Asian, and American dogs, and the mtDNA types radiating from A29 in the minimum-spanning network were found almost exclusively in East Asia (11), strongly indicating an East Asian rather than Indian origin for the dingo ancestor. The estimated time for the founding of the dingo population, ≈5,000 yr ago, fits relatively well with the archaeological record of the region, with the oldest finds of dingo being 3,500 years old and the earliest finds of dogs on nearby islands being 3,500-year-old remains on Timor (7). An East Asian ancestry ≈5,000 yr ago suggests that the dingoes may have

arrived in connection with the expansion from south China into Island Southeast Asia of the Austronesian culture, which involved domestic dogs, pigs, and chicken. According to the current theories, the expansion started ≈6,000 yr ago from Taiwan via the Philippines to Indonesia, where it was split into a westward and an eastward direction and had by 4,000 yr ago reached Timor (7, 21).

In conclusion, this study of mtDNA sequence variation among dingoes provides a number of clues from which a detailed picture of the origin and history of the Australian dingo can be derived. The dingo originated from a population of East Asian dogs. Type A29 was one of several domestic dog mtDNA types brought into Island Southeast Asia, but only A29 reached Australia. The dingo population was probably founded from a small number of animals, as the last trickle of domestic dogs through a series of bottlenecks, or even by a single chance event and has since remained effectively isolated from other dog populations. The dingoes may have arrived in connection with the expansion, starting ≈6,000 yr ago, from south China into Island Southeast Asia of the Austronesian culture. By this time, domestic dogs had existed for several thousand years (4, 11), and the present semidomestic state of the dingo can probably be attributed to a long existence as a feral animal. After >3,500 years of isolation, the dingoes represent a unique isolate of early undifferentiated dogs.

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