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An evaluation of genetic analyses, skull morphology and visual appearance for assessing dingo purity: implications for dingo conservation

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Abstract. The introgression of domestic dog genes into dingo populations threatens the genetic integrity of 'pure' dingoes. However, dingo conservation efforts are hampered by difficulties in distinguishing between dingoes and hybrids in the field. This study evaluates consistency in the status of hybridisation (i.e. dingo, hybrid or dog) assigned by genetic analyses, skull morphology and visual assessments. Of the 56 south-east Queensland animals sampled, 39 (69.6%) were assigned the same status by all three methods, 10 (17.9%) by genetic and skull methods, four (7.1%) by genetic and visual methods; and two (3.6%) by skull and visual methods. Pair-wise comparisons identified a significant relationship between genetic and skull methods, but not between either of these and visual methods. Results from surveying 13 experienced wild dog managers showed that hybrids were more easily identified by visual characters than were dingoes. A more reliable visual assessment can be developed through determining the relationship between (1) genetics and phenotype by sampling wild dog populations and (2) the expression of visual characteristics from different proportions and breeds of domestic dog genes by breeding trials. Culling obvious hybrids based on visual characteristics, such as sable and patchy coat colours, should slow the process of hybridisation.

Introduction

The dingo (*Canis lupus dingo*) is a primitive canid that arrived in Australia ~3500 years ago according to archaeological evidence (Milham and Thompson 1976). The subsequent arrival of domestic dogs (*C. lupus familiaris*) with European settlers in the 18th century has resulted in significant levels of hybridisation between the two subspecies, and this is now regarded as the greatest threat to the long-term survival of the 'dingo' (Corbett 1995, 2001). Although it is argued by some that the dingo in its pure form is rare or no longer exists (Daniels and Corbett 2003), there are intrinsic and cultural reasons for conserving the most pure form of contemporary dingoes (Fleming *et al*. 2001; Daniels and Corbett 2003). A practical method that can be used in the field to estimate the extent of hybridisation is urgently required so that hybrids below a particular threshold (e.g. $\leq\frac{1}{2}$ dingoes) can be removed from dingo populations to slow the rate of hybridisation and maintain dingo populations in as pure a state as possible.

Methods based on the analysis of genetic variation, skull morphology and visual appearance have been used independently (Newsome *et al*. 1980; Newsome and Corbett 1982; Thomson 1992; Woodall *et al*. 1996; Wilton *et al*. 1999; Wilton 2001) or coupled (Corbett 1985, 1995, 2001; Newsome and Corbett 1985; Jones 1990) to assess the extent of hybridisation with domestic dogs. However, current genetic and skull morphology methods do not allow the rapid assessment of wild dogs, and their use in the field is impractical. This is because DNA samples require laboratory analysis and skull measurements can only be reliably and accurately taken from deceased, adult animals (Corbett 2001). The use of visual characters, such as coat colour, is a practical and rapid method to apply in the field. However, the sole use of visual characters to assess the extent of hybridisation is questionable because little is known about the relationship between phenotype and the degree of hybridisation with domestic dogs. In practice, a wild dog is visually judged as a dingo or hybrid on the basis of the subjective opinion of observers, based on their prior experience. The present study evaluates the agreement in the status of hybridisation assigned to animals by genetic analyses, skull morphology and visual appearance, and also investigates the use of visual characteristics for estimating the extent of hybridisation.

Materials and methods

Study animals

Sixty wild dogs were collected from various localities throughout south-east Queensland (SEQ), Australia, in 2003–04 (Fig. 1). The

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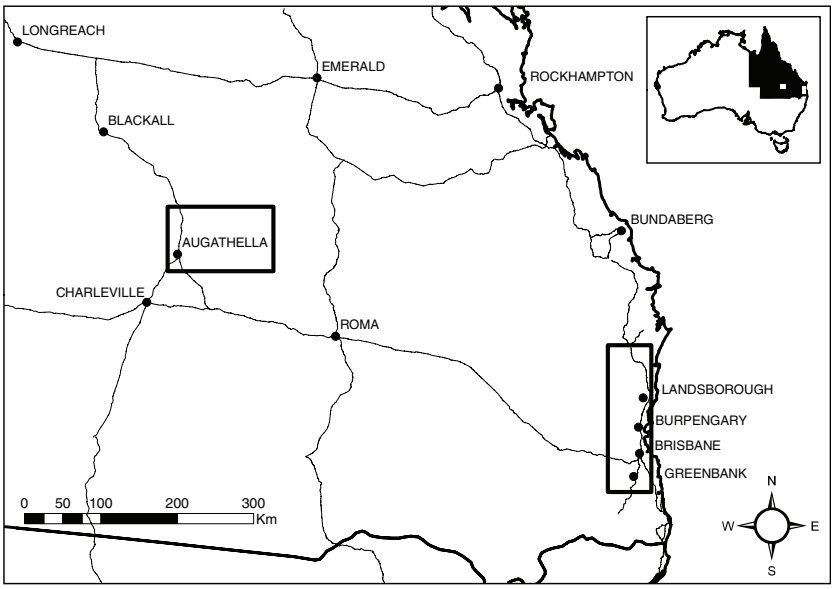


Fig. 1. Capture locations of the 100 wild dogs used for this study. Sixty animals were collected from south-east Queensland in 2003–04 and 40 animals were from western Queensland in the late 1980s.

primary collection localities were Burpengary ($n = 17$), Greenbank ($n = 12$) and Landsborough ($n = 8$). In addition, 40 skulls collected from western Queensland, north-east of Augathella, in the late 1980s were used (Fig. 1). These skulls are maintained at the Queensland Museum, Brisbane, and were included in the study to increase the likelihood of having a reasonable sample of pure dingoes for skull morphometric and genetic analyses.

Genetic analyses

An analysis of genetic variation was conducted (by A.W. and B.-L.C.) on all the animals collected for this study ($n = 100$). The samples collected for analyses were either dried tissue attached to the skulls of animals (museum samples) or ear samples from recently deceased animals that were dried in an oven overnight at ~55°C (SEQ samples). The procedures used for PCR amplification and genotyping have previously been described by Wilton (2001). The primers used were obtained from Proligo Australia and Applied Biosystems, and all the forward primers were fluoro-labelled for detection of PCR products on ABI 3730 DNA sequencers.

The 22 microsatellite loci typed were AHT103, AHT109, AHT125 (Holmes *et al*. 1993), VIASD10 (=PEZ1) (Primmer and Matthews 1993), CPH2 (Fredholm and Winter 1995), CXX109, CXX30, CXX402, CXX406, CXX410, CXX434, CXX460 (Ostrander *et al*. 1993), FH2079, FH2138, FH2175, FH2199 (Francisco *et al*. 1996), FH2247, FH2257, FH2293, FH2313, FH2346 (Mellersh *et al*. 1997), and LEI008 (Mellersh *et al*. 1994). They were chosen for their differences in allele frequencies between dogs and dingoes, some of which are diagnostic, and they have previously been used to estimate the proportion of domestic dog ancestry in dingoes (Wilton 2001; Banks *et al*. 2003). A diagnostic insertion/deletion (indel) polymorphism was also typed. M13TT is an insertion of two base pairs that occurs in the dingo but is absent from most western dogs in Australia (B.-L. J. Carlsson, J. Chiang and A. N. Wilson, unpubl. data). Such diagnostic and easily scored markers are ideal for detecting dog ancestry but require large efforts to find and characterise. Partial sequencing of the dingo genome is underway by A.W. to identify more diagnostic single nucleotide polymorphisms and indels.

The score used to summarise the test results was ‘average 3Q’, which is based on the relative probability that an animal is from a pure dingo population rather than a population of $^3/_4$ dingoes. It is the log of the probability ratio divided by the number of loci tested. Also used for assessment are diagnostic alleles for dog ancestry, i.e. those not found in a reference population of 60 dingoes assessed as pure by captive breeding history, but found in a dog reference population (90 dogs of mixed breed from pounds in Sydney region and Alice Springs) (Wilton 2001).

A scoring system (Table 1) has been developed to assign a status to each animal based on its average 3Q score and the presence of alleles that are ‘diagnostic of dog’ ancestry. Animals were assigned a dingo status if they had an average 3Q score in the

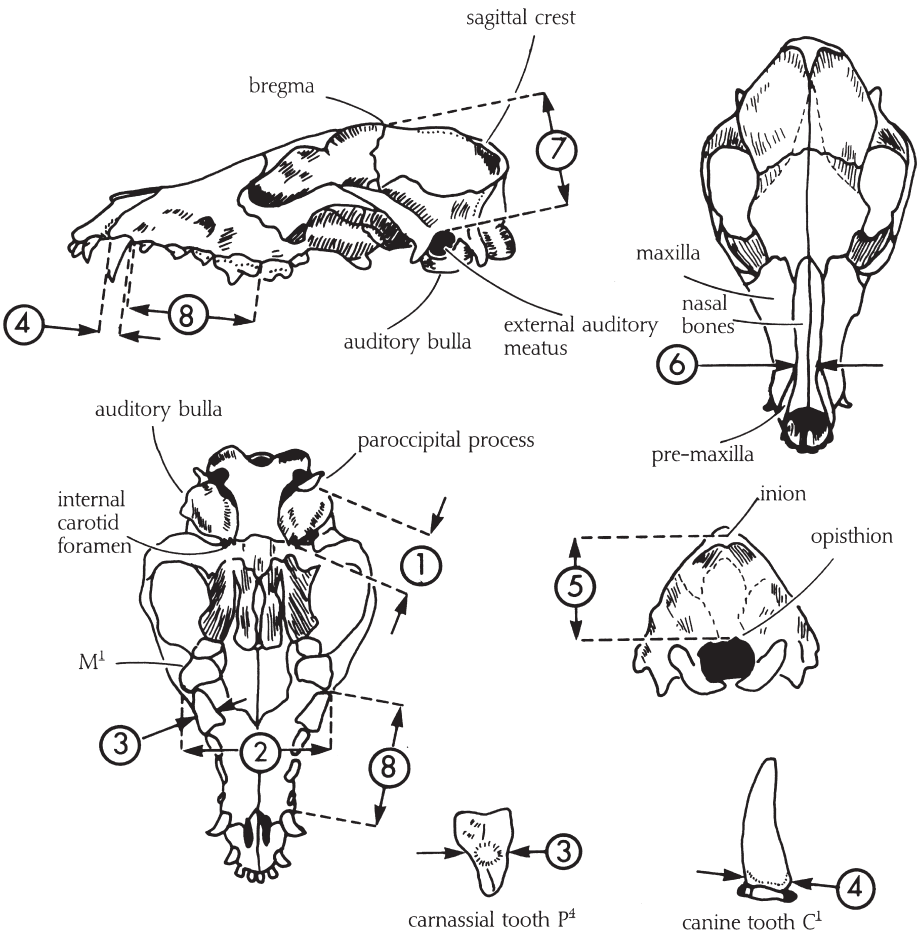
Table 1. Scoring system used to assign a dingo, hybrid or dog status to each animal based on the analysis of genetic variation

Score	Status	Average 3Q	No. of alleles ‘diagnostic of dog’ ancestry
1	Dingo	>0.1	0
2	Dingo	0.05 to 0.1	0
3	Hybrid (>75% dingo genes)	0 to 0.05	≥1
4	Hybrid (<75% dingo genes)	–0.1 to 0	≥1
5	Hybrid (<65% dingo genes)	–0.25 to –0.1	≥1
6	Hybrid (<50% dingo genes)	–0.5 to –0.25	≥1
7	Dog	<–0.5	≥1

range found for the reference dingo population (0.06–0.21) and no alleles diagnostic of dog ancestry were present. Animals were assigned a hybrid status if they had an average 3Q score greater than –0.5 (i.e. greater than the range for the reference dog population) and alleles diagnostic of dog ancestry were present. A dog status was assigned to animals with an average 3Q score less than –0.5 and alleles diagnostic of dog ancestry were present. Although the scoring system allows the identification of animals with varying proportions of dingo ancestry, all hybrid groups were pooled into the one category for analyses to complement the categories assigned by skull morphology (i.e. dingo, hybrid and dog). All loci were not successfully tested for all animals. The minimum number of loci genotyped for any animal was 14.

Skull morphology

Skull morphology assessments were conducted by Dr L. Corbett (EWL Sciences, Darwin) on all the animals collected for this study ($n = 100$) based on the methods described in Corbett (1995) (Fig. 2). This method uses eight skull



Skull measurements. Numbers in circles refer to x_1 to x_8 below.

x_1 = length of auditory bulla (measured from where it abuts the paroccipital process to the internal carotid foramen, excluding any projection on the foramen).
x_2 = maximum maxillary width (measured at about the junction of the P^4 and M^1 teeth).
x_3 = mid-crown width of the P^4 tooth (measured through the highest cusp in a lateral direction)
x_4 = basal crown length of C^1 (measured along the tooth row).
x_5 = opisthion to inion (measured from a central inion point and not including the notch in the opisthion, if present).
x_6 = width of both nasal bones (measured at premaxilla–maxilla suture).
x_7 = cranial height (measured from the upper notch of the external auditory meatus to the bregma, including the sagittal crest).
x_8 = distance between the posterior alveolar rims of C^1 – P^4.

Fig. 2. Eight skull measurements used to discriminate dingoes, hybrids and domestic dogs (Corbett 1995). All dimensions are measured in millimetres (mm).

measurements, x_1–x_8, in a canonical equation to derive a composite skull score (Y):

$$Y = 0.249x_1 - 0.261x_2 + 1.999x_3 - 1.137x_4 + 0.318x_5 + 0.457x_6 - 0.205x_7 + 0.136x_8 - 3.717$$

A domestic dog, hybrid or dingo status can then be assigned to each animal on the basis of the composite skull score and the 95% confidence limits (CL) specified for dingoes by Corbett (1995). A dingo status was assigned to animals with a skull score $\geq$1.271 and all eight skull measurements within the 95% CL for dingoes. Animals with a skull score $\geq$1.271 and any of the eight skull measurements outside the 95% CL, and animals with a skull score between –1.393 and 1.270 were assigned a hybrid status. A domestic dog status was assigned to animals with a skull score no higher than –1.394 (Corbett 1995). Six of the skulls in our study were damaged, and the skull scores for these animals were calculated using alternative equations (Newsome and Corbett 1985, table 1, eqns 2 and 15).

Visual appearance

There is no established method or strategy based on visual appearance to assess hybridisation. Therefore a survey of experts on wild dogs was conducted to determine the characters that are commonly used in the visual assessment of hybridisation. Thirteen survey participants were selected on the basis of their extensive experience with dingoes in the field. They included representatives from state and local government organisations and dingo conservation groups across Australia (10 from Queensland, two from New South Wales, one from Western Australia). All participants were provided with the same photographs of each of the 56 sampled SEQ animals, without knowing the results of genetic analyses or skull morphology. No photographs were available for four SEQ animals or the 40 museum animals. A subset of 13 animals was selected as a minimum for the participants to complete if the time they had available to complete the survey was limited. The 13 animals selected exhibited the full range of visual characters observed in the original 56 animals, and some animals were used because, although the authors of this paper considered them to look similar, their status assigned by genetic analyses and skull morphology differed. Eight of the 13 survey participants assessed the status of all 56 animals.

The participants were requested to nominate the visual characters that they used to assess the status of an animal and indicate on a five-point scale whether they considered each character to be: strongly dingo-like; dingo-like; neutral; dog-like; or strongly dog-like. The most common characters used in assessments included coat colour, the presence/absence of white points, tail form and brushiness, the presence of sable, and the presence of ticking or 'spotting' in the coat. Head and body conformation and the presence of floppy ears were auxiliary characters used by some of the survey participants. The participants were not provided with character descriptions so that they would select characters based on their own prior knowledge as these would be what they currently use in the field. A brief description of the main characters and their use for discriminating dingoes from hybrids and other domestic dogs is provided in Table 2.

The participants were also requested to assign each animal a status (dingo, $\frac{3}{4}$, $\frac{1}{2}$, or $\frac{1}{4}$ dingo, or dog) based on their assessment of these characters. However, as was the case with the genetic analyses, all hybrid groups were later pooled into the one category for analyses to complement the categories assigned by skull morphology. The median status assigned to each animal by survey participants was used as the representative score of visual appearance in subsequent analyses. The median value was used rather than the mean or mode so that the estimated status of hybridisation was not distorted as much by outlying scores.

Table 2. Description of visual characteristics used in the survey and their use for discriminating dingoes from hybrids and other domestic dogs

Character	Description
Coat colour	Although ginger is the most commonly accepted coat colour of 'pure' dingoes, black-and-tan, black, and white coat colours may also be considered characteristic of dingoes. Hybrids are generally distinguished from dingoes by rare or unusual coat colours, such as sable (see below), patchy (typically white animals with large ginger or black patches) or brindle (banding in the coat) (Jones 1990; Corbett 1995; Fleming *et al*. 2001).
Sable	Sable animals have dark hairs that form a prominent dorsal stripe from the head to the tail, as seen in German shepherd dogs. Sometimes the dark hairs may extend down onto the shoulders and sides of the chest (Newsome and Corbett 1985; Jones 1990; Corbett 1995; Fleming *et al*. 2001). The presence of sable indicates hybridisation with domestic dogs.
Ticking	Ticking is a term to describe spotting in the coat, as often seen in Australian cattle dogs. The presence of ticking in the coat indicates hybridisation with domestic dogs, and is a useful character when the coat colour of an animal otherwise indicates that it is a pure dingo (Corbett 1995).
White points	Dingoes are generally thought to have five white points: four white toes, feet or socks and a white tail tip (Thomson 1992; Corbett 1995). However, the amount of white varies considerably between individuals (Jones 1990) and, in some cases, there may be so few white hairs that they cannot be seen from a distance. The absence of white points indicates hybridisation.
Tail form and brushiness	An excessively long tail (relative to body length) and/or a non-bushy tail are indicative of hybridisation (Jones 1990; Corbett 1995).
Head and body conformation	Hybrids that result from crosses between dingoes and large domestic dog breeds, such as Dobermans, can be identified by their large, heavy body and broad heads (Corbett 1995).
Floppy ears	Dingoes generally have short erect ears, and ears that do not stand erect (flop) indicate dog ancestry (Sanderson 1981; Corbett 1995).

Statistics

A two-sided Exact Test was used to determine the relationship between genetic analyses, skull morphology and visual appearance by pair-wise comparisons. This test provides only a *P*-value statistic ($\alpha = 0.05$), which is the sum of probabilities for all possible tables with the same row and column totals that have a probability of occurrence no greater than that of the observed table. In addition, a Cramer's V Test was used to measure the association between the status of hybridisation assigned to animals (i.e. dingo, hybrid or dog) by genetic analyses, skull morphology and visual appearance. These data were also analysed by a pair-wise comparison of methods, with values ranging from 0 (no association) to 1 (perfect association). Both the Exact and Cramer's V Tests were applied to the data using the FREQ procedure in SAS (ver. 8.2).

Results

Comparison of genetic analyses, skull morphology and visual appearance

An overall comparison of the status of hybridisation assigned to 56 of the SEQ animals by genetic analyses, skull morphology and visual appearance found that 39 (69.6%) of these animals were assigned the same status of hybridisation by all three methods. In addition, 10 (17.9%) animals were assigned the same status by genetic analyses and skull morphology, four (7.1%) by genetic analyses and visual appearance, and two (3.6%) by skull morphology and visual appearance. One animal (1.8%) was given a different status by all three methods. The status of hybridisation assigned to the 40 museum animals by genetic analyses and skull morphology agreed for 26 (65%) cases. Figure 3 shows the status of hybridisation categories assigned to each animal by genetic analyses, skull morphology and visual appearance for both the SEQ and museum animals.

The two-sided Exact Test showed a significant relationship ($P = 0.021$) between genetic analyses and skull morphology, but not between genetic analyses and visual appearance ($P = 0.622$) or skull morphology and visual appearance ($P = 1.000$) (Table 3). These results reflect the moderate association between the status categories assigned to animals by genetic analyses and skull morphology (Cramer's V = 0.393), and the weak association of status categories between genetic analyses and visual appearance (Cramer's V = 0.154) and skull morphology and visual appearance (Cramer's V = 0.154) (Table 3).

Assessment of visual appearance as a method for estimating purity

The most common character used by survey participants to visually assess the extent of hybridisation was coat colour (95.6%). The other characters used were the presence/absence of white points on the feet and tail tip (88.8%), the extent of brushiness and shape of the tail (70.1%), the presence of sable (65.4%), and the presence of ticking or 'spotting' in the coat (40.6%). Head and body conformation and the presence of floppy ears were auxiliary characters used by some of the survey participants. However, conformation was difficult for survey participants to assess because the photographs were of unconscious animals and only one of the animals had floppy ears. Variability in the assignment of scores for some visual characters was observed. For example, the same animal was assigned dog-like colouration and dingo-like colouration by different participants. In addition, the score assigned to each visual character did not always reflect the overall status assigned to the animal. Nonetheless, no consistent differences could be detected between participants in the scores assigned to each visual character or the status of an animal.

Table 4 presents the status of hybridisation assigned to each of the 56 SEQ animals by genetic analyses, skull morphology and visual appearance within each of the coat colours. The frequency of pure dingoes did not appear to differ markedly between each of the methods. For example, genetic analyses detected only 22.2%

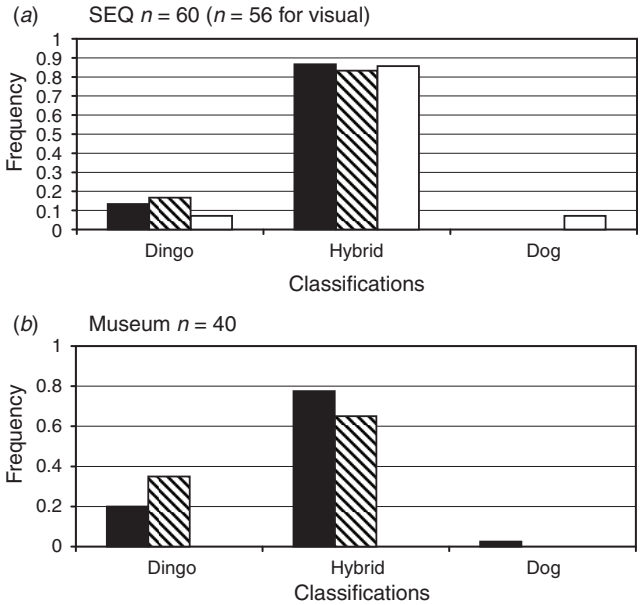


Fig. 3. Number of individuals assigned to each status category by genetic analyses (solid black bars), skull morphology (striped bars) and visual appearance (solid white bars) for (*a*) the 60 SEQ and (*b*) the 40 museum samples. Only 56 of the SEQ animals were used in the visual assessment method.

Table 3. Percentage agreement and association for the status of hybridisation assigned to the 56 SEQ animals by genetic analyses, skull morphology and visual appearance

	% agreement	No. of dingoes	No. of hybrids	No. of dogs	Exact test	Crammer's V
Genetic analyses and skull morphology	87.5	3	46	0	0.021	0.393
Genetic analyses and visual appearance	76.8	0	43	0	0.622	0.154
Skull morphology and visual appearance	73.2	0	41	0	1.000	0.154

Table 4. Status of hybridisation assigned to the 56 SEQ animals within each of the coat colours, as determined by genetic analyses, skull morphology and visual appearance

	Ginger	Sable	Black-and-tan	Black	Patchy	Piebald	Brindle	Total
n	18	17	10	5	3	2	1	56
Genetic analyses								
Dingo	4	0	1	0	0	1	0	6
Hybrid	14	17	9	5	3	1	1	50
Dog	0	0	0	0	0	0	0	0
% Pure	22.2	0.0	10.0	0.0	0.0	50.0	0.0	
Skull morphology								
Dingo	3	1	2	0	0	0	1	7
Hybrid	15	16	8	5	3	2	0	49
Dog	0	0	0	0	0	0	0	0
% Pure	16.7	5.9	20.0	0.0	0.0	0.0	100.0	
Visual appearance								
Dingo	3	0	1	0	0	0	0	4
Hybrid	15	17	9	4	2	0	1	48
Dog	0	0	0	1	1	2	0	4
% Pure	16.7	0.0	10.0	0.0	0.0	0.0	0.0	

of ginger animals as dingoes, and skull morphology and survey participants 16.7%. However, the three ginger animals identified as dingoes by skull morphology were also identified as dingoes by genetic analyses, but were different to the three ginger animals visually identified as dingoes by the survey participants. Except for the ginger and sable coat colours, the sample sizes were small and may not have represented the coat colours well in relation to the purity of animals within the three methods. Furthermore, some of the ginger-coloured animals exhibited ticking or other marks in the coat.

Of the 100 animals in this study, the status of hybridisation assigned to animals by genetic analyses and skull morphology was in agreement for 76% of cases and identified eight dingoes and 68 hybrids. However, Corbett (1995) recommends that the most confident assessment that an 'unknown' animal is a pure dingo requires: (1) a total skull score in the range for dingoes; (2) all eight individual skull measurements within the 95% confidence limits for dingoes; and (3) a coat colour that is ginger, black, black-and-tan, or white without any oddities. The first two criteria were used during the status-assignment procedure for skull morphology and were already satisfied. When coat colour was considered in the assignment procedure for skull morphology, genetic analyses and skull morphology agreed for 80% of cases but identified only five dingoes. This is because animals that were originally assigned a dingo status by skull morphology were reassigned a hybrid status if their coat colour was not characteristic of dingoes (e.g. sable or brindle). The agreement between genetic analyses and skull morphology increased by 4% because seven of the 10 dingoes that were downgraded to a hybrid status were then in agreement with the hybrid status assigned by genetic analyses. Six ginger and two black-and-tan animals whose dingo status was assigned according to skull morphology had been reassigned a hybrid status by genetic analyses. The five animals that had been assigned a dingo status by genetic analyses and maintained their dingo status by skull morphology all had a ginger coat.

Discussion

There was relatively strong agreement between the status of hybridisation assigned to animals by genetic analyses, skull morphology and visual appearance. Skull morphology and genetic characteristics are both able to discriminate between dingoes, hybrids and domestic dogs. However, limitations of these methods are that they have difficulty discriminating between purebred dingoes and hybrids that are backcrossed to dingoes, and the procedures cannot be applied to live animals in the field (Woodall *et al*. 1996; Daniels and Corbett 2003; Elledge *et al*. 2006). Furthermore, skull morphology uses a canonical equation that was developed on animals collected in central Australia (Newsome *et al*. 1980). The range of values used for dingoes in this method does not account for geographic variation that may (or may not) exist between populations in Australia. In addition, repeatability of measurements is important for an accurate assessment of skull morphology. For example, Rasmussen *et al*. (2001) found that morphological measurements of geese were more precise with experienced individuals and that there was also less misclassification than with inexperienced individuals. Dr Corbett, who developed the 'skull morphology' method, made the measurements for this study to avoid any potential bias of inexperienced personnel.

The dingo status assignment used in genetic analysis is based on a limited number of markers that do not cover the entire genome. Consequently, low levels of domestic dog ancestry will not always be detected and a score in the range of the dingo reference population does not necessarily mean that the animal is a dingo, but rather that there is no strong evidence of domestic dog ancestry in the alleles tested. Genetic assessment of status is more

reliable when a larger number of markers are used, which subsequently also increases the cost. For example, Vaha and Primmer (2006) found that 12–24 loci were required to detect F_1 hybrids but at least 48 loci were required to separate backcrosses from purebred parents. For efficient extraction of information with minimal cost, 23 markers were tested in this study. Moving from microsatellite loci to single-nucleotide polymorphisms and indels would give genetic analysis an advantage as large numbers could be typed cheaply to provide the power to detect low levels of dog ancestry. In the future, devices developed for use in personalised medicine (Cyranoski 2005) will be able to type hundreds of such markers simultaneously and may make the technique applicable in the field.

The use of visual characters to assess the extent of hybridisation in the field would greatly facilitate dingo conservation, but at present the method is poorly defined and assessments are subjective, depending on the experience of the observer (Elledge *et al*. 2006). The use of photographs for assessing the extent of hybridisation in the present study precluded the use of potentially important cues that the survey participants might normally use in the field, such as behaviour, stance, and body conformation. It is possible that visual assessments in the field are more accurate than our results show. We recommend that future research focus on four main themes: (1) define and interpret phenotypic characters of dingo populations; (2) determine the type of crosses and backcrosses that lead to the expression of hybrid characters and those that do not; (3) substantiate what domestic dog characters persist in hybrids; and (4) determine the number of backcross generations to dingoes that leads to the suppression of hybrid characters.

Understanding the process of domestication and the introgression of domestic genes into wild populations are for assessing phenotypic changes related to hybridisation. For example, a long-term study on the domestication of red foxes (silver morph – *Vulpes vulpes*) reported notable character changes, including the loss of pigmentation in the coat (e.g. piebald), floppy ears, rolled tails, and reduced or increased body size and proportions (Trut 1999). Furthermore, Barilani *et al*. (2005) also commented that the introgression of domesticated Japanese quail (*Coturnix japonica*) genes into wild common quail (*Coturnix coturnix*) populations might affect the expression of characters such as body size and feather colours. Data from our survey indicate that hybrids are more readily positively identified through visual characters than are dingoes, implying that there is a greater knowledge of visual characters that traditionally indicate domestic dog ancestry, such as floppy ears and the presence of ticking, rather than what characters dingoes may actually exhibit. A range of characters that are expressed by dingoes can be determined by the examination of wild and captive populations that are genetically proven as 'pure'. It is important that each character is described in detail and the extent of its expression on the body is interpreted. For example, Corbett (1985) found that only 5% of wild dogs had a large white tail tip while many had only a few white hairs. Although the amount of white varies considerably between individuals (Jones 1990), it is not known which is more characteristic of dingoes.

A breeding trial, such as that conducted by Newsome and Corbett (1982), is strongly recommended to obtain hybrids with known proportions of dingo ancestry. Although the pelts and skulls of animals from this study are available for further assessment, we recommend that a more extensive experiment also be conducted to better understand the effect of genetics on the expression of visual characters. This is because the experiment by Newsome and Corbett (1982) obtained only $\frac{1}{4}$, $\frac{1}{2}$, $\frac{3}{4}$ and $\frac{7}{8}$ dingoes from crosses with the Australian cattle dog, even though four other domestic dog breeds were used to obtain hybrids with 50% dingo ancestry. Furthermore, three of the eight parental dingoes were found to be intermediate to dingoes and domestic dogs on the basis of skull morphology. This suggests that they were either hybrids, or demonstrated morphological effects of domestication, or represented extreme values in the range of dingoes (Newsome and Corbett 1982).

Parental dingo stock would ideally be animals from remote locations, such as central Australia, with no or negligible domestic dog genes confirmed by genetic analysis. Although the museum specimens of wild dogs in this study were predominately classified as hybrids, 77.5% were actually dingoes or hybrids with >75% dingo genes, compared with only 33.3% of SEQ wild dogs in these two categories. The exclusion of long-term captive animals as parental dingo stock and the longevity of experimental animals in captivity should also be considered as captive populations diverge in phenotype from their wild counterparts over generations in captivity (Trut 1999; McPhee 2004; Trut *et al*. 2004). F_1 hybrids from dingoes and non-dingo-like domestic dogs, F_1 hybrids backcrossed to domestic dogs, and F_2 hybrids can be distinguished from pure dingoes by their rare or unusual colouration and body proportions (Newsome and Corbett 1985; Jones 1990; Corbett 1995). For example, hybrids with German shepherd ancestry may exhibit heavy sable and a large, heavy body atypical of dingoes.

Experiments with dingo and dingo-like domestic dogs are particularly important for detecting subtle character differences. Domestic dog breeds such as the Australian cattle dog and Australian kelpie are similar to dingoes in colouration and body proportions, and resulting hybrids can be extremely difficult to distinguish from pure dingoes by the sole use of phenotypic characters (Newsome and Corbett 1985; Corbett 1995). Although characters such as ticking or floppy ears could be used to detect hybrids, it is not known at what proportion of dog ancestry or breed these are expressed, apart from Corbett's (1995) observation of ticking in F_1 hybrids of dingo and Australian cattle dog. Nonetheless, do characters such as ticking, floppy ears and body proportions become suppressed when hybrids with various proportions of domestic dog genes are backcrossed to dingoes?

The criteria described in Corbett (1995) for the most confident assessment of dingo purity on the basis of skull morphology (phenotype) states that an animal must have either a ginger, black, black-and-tan, or white coat without any oddities. This definition was further refined by Corbett (2001) to animals with a ginger coat only. Elledge *et al*. (2006) suggested that the latter criterion was too strict in that all animals with a coat colour characteristic of dingoes other than ginger were given a hybrid status. However, results from the present study may support the criteria described in Corbett (2001) as only ginger-coloured animals maintained their dingo status when results from

genetic analysis and skull morphology were compared, as determined by the criteria in Corbett (1995). Nonetheless, wildlife managers must determine what they are willing to accept as a 'pure' dingo in conservation areas, as the culling of all animals that do not exhibit all traits characteristic of dingoes may result in severe demographic loss and/or genetic bottleneck. For example, Miller *et al*. (2003) discusses a strategy for reducing the demographic loss of red wolf (*Canis rufus*) genes by tolerating hybrids with a small proportion of coyote (*Canis latrans*) genes (e.g. <25%). There is potential to apply a similar strategy to dingo populations in conservation areas.

We conclude by recommending that future research should be conducted to determine (1) the relationship between genetics and phenotype by sampling wild dog populations extensively and (2) the expression of visual characteristics in relation to the proportion and breed of domestic dog genes through experimental breeding trials. This knowledge will be useful for developing a more reliable visual method that will allow wildlife managers to identify and promptly remove hybrids from dingo populations in the field. Until then, culling obvious hybrids on the basis of visual characteristics, such as sable and patchy coat colours, should slow the process of hybridisation.

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