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Guest Editorial

Analysis of the canine genome and canine health: Bridging a gap

'*All meanings, we know, depend on the key of interpretation.*'
George Eliot, *Daniel Deronda.*

Words can so easily carry different meanings. Since the original screening of the BBC TV documentary '*Pedigree Dogs Exposed*' in 2008, some of the words written by two of us (FN and CW) have been perceived as implying that inbreeding 'does not matter', i.e. the mating of close relatives is not harmful. This misperception was one reason behind the writing of the recent Personal View in *The Veterinary Journal* by PB and DS, from which the quotation 'does not matter' is taken (Bateson and Sargan, 2012). Publication of the Personal View led to a lengthy but interesting correspondence between all four authors, during which it became clear that whilst we differed in our emphasis on a number of points of detail, we are all four in agreement on the main issues as they affect canine genetic health.

We have now had time to consider various ways to bridge the gap that might be seen to stand between us, and eventually we decided that the best way forward would be to prepare a piece which all four authors could co-author and endorse. With the generous indulgence of the Editor of this journal we now present our joint views. In so doing, we wish to provide in particular an unambiguous statement that FN and CW have always been, and are still now, in complete agreement with PB and DS (and every geneticist of whom we are aware) that matings of dogs (indeed all domestic animals) should be arranged so as to minimise the level of inbreeding in the offspring.

Early in their Personal View, Bateson and Sargan (2012) provided the following quote from Nicholas and Wade (2011): '*... direct estimates of the extent of canine genetic diversity indicate that dog breeds retain a very high proportion of genetic diversity. In other words, in terms of the extent of genetic diversity, dogs are far, far closer to humans than to inbred lines.*' In this quotation FN and CW were using the words '*inbred lines*' in the sense of lines with a coefficient of inbreeding (CoI) of unity, and hence heterozygosity of zero, i.e. lines having zero genetic variation. Such lines are often called isogenic lines, and we will use these words when referring to such lines in the rest of this Guest Editorial. For this intended meaning, PB and DRS would certainly endorse the above quotation from Nicholas and Wade (2011).

It was initially the ambiguities of words that came between the two pairs of authors: PB and DRS interpreted 'inbred lines' as referring to populations such as the well-studied human line of the Spanish Habsburg royal dynasty (Alvarez et al., 2009) or isolated sub-populations of other species that have been judged to need genetic restoration now, such as those on the American Livestock Breeds Conservancy '*Critical*'[1] or UK Rare Breeds Trust '*Watch*'[2] lists. This was the interpretation that drove the Personal View of Bateson and Sargan (2012). Since the implications of this interpretation have already been discussed by Bateson and Sargan (2012), we shall not pursue this interpretation any further.

In endorsing the quotation from Nicholas and Wade (2011), with the meaning of 'inbred lines' clarified, all four authors acknowledge that such (isogenic) lines typically do have some genetic variation, as new variants are created by mutation and enter gametes in every meiosis. However, standard breeding practices with isogenic lines maintain this 'mutational variance' at a very low level, and for all practical purposes (including the context of this Guest Editorial), isogenic lines can be regarded as having zero genetic variation.

It is important to note that the endorsed quotation is not claiming that dogs are like humans. The results of many human and canine pedigree analyses show that, typically, dogs are more inbred than humans. However, one of the very important aims of the quote is to highlight the fact that even though dogs are, typically, more inbred than humans, they have CoIs that are far closer to those found in humans (i.e., ranging mainly from 0% to 6.25%,[3] but with documented cases as high as 25.4%[4]) than to the inbreeding coefficient of isogenic lines (in effect, 100%). For example, among the 2.1 million dogs in 10 numerically strong breeds included in the large UK canine pedigree analysis by Calboli et al. (2008), the average CoI of breeds accumulated over 6–7 generations ranged from 2.4% (Labrador retriever) to 7.3% (Rough collie). The highest levels of documented inbreeding in dogs of which we are aware are all consistent with the endorsed quotation: the Nova Scotia Duck Tolling Retriever (average CoI = 26%, with 13 generations average pedigree depth; Mäki, 2010); Irish setters (average CoI = 16% at 10 generations, 29% at 20 generations and 33% at 30 generations; Urfer, 2009); Polish hounds (average CoI = 37%; Głażewska, 2008).

Some dog breeds are considered by their registering bodies to be vulnerable and it is important to consider whether breeds are genetically endangered. A relevant criterion for an endangered breed has been provided by the European Association for Animal Production-Animal Genetic Data Bank (EAAP-AGDB), which classifies a breed as endangered if it is likely to experience an increase in

[1] See: http://www.albc-usa.org/cpl/wtchlist.html.
[2] See: https://www.rbst.org.uk/watchlist.pdf.
[3] As explained by Bittles (2002): 'Globally, the most common form of consanguineous marriage [in humans] is between first cousins, who are predicted to have 12.5% of their genes in common ... equivalent to a coefficient of inbreeding (*F*) of 0.0625 [in their offspring]'.
[4] Charles II of Spain (Alvarez et al., 2009).

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the level of inbreeding of 26% or more over 50 years (Simon and Buchenauer, 1993; FAO, 2007). Noting that effective population size (N_e) can be calculated from the classic formula (Falconer and Mackay, 1996) as $1/(2\Delta F)$, where ΔF is the increase in inbreeding per generation, and taking the average generation interval of pedigree dogs to be around 4 years (Shariflou et al., 2011), this criterion equates to $N_e = 24$ for dogs. On this genetic criterion, one of the 10 breeds studied by Calboli et al. (2008), the Greyhound, is seen as endangered. KC registered Greyhounds for which pedigree information was available in that study form only a very limited section of the total Greyhound population and there is a separate racing population. Nonetheless it is the only one of the 10 breeds in the study that is on the UK Kennel Club's list of 24 'vulnerable native breeds'. In the case of the Greyhound though, the very long history of the breed may have led to considerable purging of disadvantageous alleles. None of the 48 French breeds studied by Leroy et al. (2009) for which N_e could be calculated, would be regarded as endangered by the EAAP-AGDB criterion. These 48 breeds were selected as being representative of French dog breeds, and included the 33 most numerous French breeds.

In addition to their endangered category, the EAAP-AGDB also has a 'minimally endangered' category, which converts to a threshold for dogs of $N_e = 39$. Among the UK breeds studied by Calboli et al. (2008), one further breed, the Rough collie, falls below this criterion, with $N_e = 33$. Of the 48 French breeds studied by Leroy et al. (2009), two fall below the criterion, namely the Berger des Pyrénées ($N_e = 30$) and Braque Saint-Germain ($N_e = 29$). Reviewing all available estimates of N_e in dogs (including the studies just mentioned), Leroy (2011) reported the median N_e across 66 dog breeds to be approximately 93, which (as he noted) is comparable to other domestic animal species.

Some people would argue that the EAAP-AGDB criteria should be more demanding, i.e. that a loss of 26% of genetic variation over 50 years is far more than should be tolerated. Leroy (2011), for example, expressed concern for any breed with N_e less than 50, which corresponds to a loss of genetic variation of 1% per generation, which translates to around 12.5% loss in dogs over 50 years. The criterion, which was first suggested by Franklin (1980), was also cited by Bateson and Sargan (2012) as being '*the short term minimum rule of thumb*'. A further four breeds studied by Calboli et al. (2008) fall at or below the criterion. So in the UK at least, some breeds are losing genetic variation at a rate that would concern some geneticists. Other geneticists are heartened by the fact that populations with N_e substantially lower than 50, or even lower than the EAAP-AGDB minimally endangered ($N_e = 39$) or endangered ($N_e = 24$) criteria, can continue to exist in the long term, and can also continually respond to directional selection.

Maintenance of breeding populations in the long term is possible with small N_e providing there is high fecundity, so that large numbers of sick individuals can be discarded from populations. This process of purging allows the production of healthy individuals, but only at high cost to the individuals discarded from the programme because of morbidity: a cost that is hard to justify in a companion animal species.

Another important parameter of genetic variation is breed-average heterozygosity estimated from microsatellite markers. Estimates of this parameter for dog breeds range from 0.39 to 0.76 across 28 USA breeds (Irion et al., 2003); 0.40 to 0.77 across 61 French breeds (Leroy et al., 2009), and 0.47 to 0.75 across 13 of the most numerous UK dog types (pedigree breeds and non-pedigree individuals) (Mellanby et al., 2012). Comparable human estimates range from 0.50 to 0.78 (Rosenberg et al., 2002). The conclusion from these results is that the most polymorphic dog breeds have levels of microsatellite heterozygosity similar to the most polymorphic human populations, but the least polymorphic dog breeds have lower heterozygosities than the least polymorphic human populations. Overall, these results indicate that the microsatellite heterozygosity picture for dogs is not as good as for humans. Importantly, however, the results are consistent with the endorsed quotation, i.e. dogs have average microsatellite heterozygosities closer to humans than to isogenic lines.

The fact that dogs have considerable genetic diversity does not in any way undermine the need for matings to be arranged to minimise the inbreeding of offspring. On the contrary, the greater the genetic diversity, the greater will be the benefits of arranging matings so as to minimise the inbreeding of offspring! To express this another way: if dogs really had levels of heterozygosity/genetic diversity more akin to isogenic lines than to humans, there would be little point in recommending that matings be planned so as to minimise inbreeding of offspring because the closer dogs get to being an isogenic line the more genetically alike are *all* members of a breed, and the less heterozygosity/genetic diversity there is to conserve.

In their Personal View, PB and DRS also interpreted 'genetic diversity' in terms of haplotype diversity (Bateson and Sargan, 2012). All of us agree that further systematic investigation of haplotypic diversity within and among breeds and exploration of the impacts of breeder selective practices on haplotypes are worthwhile.

In our correspondence, we also debated the nature of the canine single-nucleotide polymorphism (SNP) arrays and how the selected SNPs represent variation in the dog population. CW points out that although the arrays she refers to in Wade (2011) were subsequently marketed as tested for polymorphism against >10 breeds, at the time the array was used in the study of Karlsson et al. (2007), it could not have been tested for SNP polymorphism against a range of breeds. Hence in that study it was not an array of SNPs known to be polymorphic in the widest number of breeds and so can be taken to be more representative of the level of polymorphism of random SNPs across breeds. We all accept this.

A number of other quality criteria were used in SNP choice of which only one is relevant here. Where possible, SNPs were not Boxer–Boxer SNPs (i.e., the strategy favoured SNPs demonstrating a difference between non-Boxer and Boxer). SNP-discovery breeds including the Boxer are expected to be more polymorphic on the arrays than non-discovery breeds because of inevitable ascertainment bias — some of the chosen SNPs may have inadvertently been 'private' (breed-specific because they are new mutations) within the breed of discovery (Clark et al., 2005). All four authors agree that the array-based work on breed polymorphism presents one of the clearest and simplest bases for comparison between breeds of levels of retained heterozygosity, although we still differ in how to interpret this evidence in the light of other calculations that can be made from the same molecular data. The reasons for these differences are, first, the weight placed by PB and DRS on runs of homozygosity and haplotype loss as measures of genetic diversity (Boyko et al., 2010), and, second, the importance they place on considering those breeds and particularly those individuals that have below average heterozygosity (Bateson and Sargan, 2012).

Nonetheless, we are all agreed that current levels of genetic variation within individual dog breeds are lower than amongst the whole canine population. Not surprisingly, exactly the same is true in humans, for whom there has been a '*dramatic reduction in genetic diversity within populations living outside of Africa*' due to repeated bottlenecks (the so-called 'serial founder effect') (Henn et al., 2012). As has been made clear by both CW and FN on many occasions, arranging matings so as to minimise the relationship of parents is an important strategy for maximising the extent to which genetic variation is retained within a breed (see, for example, Wade, 2011; Nicholas, 2010, p. 242). Furthermore, as explained by Nicholas and Wade (2011), minimising the relationship between parents is also the best available strategy for minimising

the chance of breeding offspring with inherited disorders: '*Inevitably, the mating of relatives increases the risk that an offspring gets two copies of one of the harmful recessive alleles carried by the common ancestor, and, consequently, is affected by the disorder. The smaller the number of generations back to the common ancestor, the greater is the risk that a descendant will have a disorder carried by the common ancestor. This biological reality applies to any ancestor, irrespective of how successful and/or famous he/she was*'.[5]

All four of us strongly endorse the latter quotation and, consequently, we strongly recommend that matings should be arranged so as to minimise the inbreeding of offspring (i.e. to minimise the relationship between parents), because this is the most effective practical strategy to decrease the chance of producing offspring with inherited disorders and to conserve heterozygosity/genetic diversity in individual dogs.

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[5] For similar statements, see Nicholas (2010) (in particular, pp. 242, 302), and the University of Sydney website http://sydney.edu.au/vetscience/research/disorders/faq.shtm.